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RESPIRATORY PROCESSES AND CARBON BALANCE IN GRAPEVINES: ENVIRONMENTAL AND GENOTYPE EFFECTS

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Nosotros,

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CERTIFICAMOS:

Que el presente trabajo titulado "RESPIRATORY PROCESSES AND CARBON BALANCE IN GRAPEVINES: ENVIRONMENTAL AND GENOTYPE EFFECTS" presentado por Esther Hernández Montes para optar al TÍTULO universitario oficial de DOCTORA por la Universitat de les Illes Balears dentro del programa de doctorado de Biología de las Plantas en condiciones Mediterráneas, se ha realizado bajo nuestra dirección.

Revisado el presente trabajo, autorizamos su presentación para que pueda ser juzgada por el tribunal correspondiente.

Palma de Mallorca, 30 de Mayo de 2017

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Esta Tesis ha sido desarrollada con la ayuda de un programa de formación de personal investigado (FPI). Los resultados obtenidos en esta Tesis ha resultado en los siguientes artículos:

1. **Hernández-Montes E.**, Tomás M., Escalona J.M., Bota J., Medrano H. (2017). Leaf growth rate and nitrogen content determine respiratory costs during leaf expansion in grapevines. *Tree Physiology* (submited).
2. **Hernández-Montes E.**, Tomás M., Escalona J.M., Medrano H. (2017). Effect of genotype and plant water status on the whole cluster respiration throughout ripening in grapevines (Vitis vinifera L.) (In process).
3. **Hernández-Montes E.**, Escalona J.M., Tomás M., Medrano H. (2017). Influence of water availability and grapevine phenological stage on the spatial variation in soil respiration. *Australian journal of grape and wine research* 23(2): 273-279.
4. **Hernández-Montes E.**, Tomás M., Escalona J.M., Martorell S., Sancho P., Bota J., Medrano H. (2017). Effect of genotype and plant water status on carbon balances and respiratory losses in grapevines (Vitis vinifera L.) (In process).

ÍNDICE

CONTENTS

RESUMEN

La importancia de la respiración en el balance de carbono de las plantas es bien conocida, pero su estudio en vid (*Vitis vinifera* L.) es todavía escaso. Por ello, hay una necesidad de estimar con precisión el efecto de las condiciones ambientales, del genotipo y de las prácticas agronómicas sobre el balance de carbono de la vid. La mayoría de estudios han determinado el balance de carbono en vid a partir de la biomasa producida o del intercambio de CO_2 a nivel de planta entera. Sin embargo, los trabajos que tienen en cuenta las pérdidas respiratorias de cada uno de los órganos son escasos. Además, el cambio climático tiene un efecto sobre la fenología y el crecimiento y supone un incremento de la actividad respiratoria de la planta debido al aumento de temperatura. Por ello, se planteó esta Tesis con el objetivo de determinar las pérdidas por respiración de diferentes órganos de la planta a lo largo de su fenología, así como sus variaciones ambientales y genéticas en condiciones realistas de cultivo. En base a estas determinaciones, se han calculado los balances de carbono en vid y las variaciones por causas genotípicas (cultivares Garnacha y Tempranillo) y edafo-climáticas (riego y sequía). Los resultados mostraron pérdidas respiratorias de los órganos de la parte aérea (hojas, tallos y frutos) en torno al 20-30%, representando entre un 7-8% y un 9-11% de carbono total fijado por la fotosíntesis en riego y sequía, respectivamente. La raíz fue el órgano con registró mayores pérdidas respiratorias en relación al total fijado por la fotosíntesis (25-30%), obteniendo los mayores valores las plantas regadas. Así mismo, el riego afectó los valores integrados de fijación y pérdida de carbono debido a la mayor materia seca acumulada. Los resultados mostraron la importancia de los procesos respiratorios, así como su variabilidad a lo largo de la fenología, mostrando un fuerte efecto del genotipo en la respiración de los órganos de la parte aérea. La consecuencia de estas diferencias en el balance de carbono se reflejó en diferencias en la acumulación de biomasa, siendo Tempranillo el cultivar con mayor biomasa vegetativa (hojas y tallos) y Garnacha mayor biomasa de los órganos reproductores y órganos permanentes.

SUMMARY

The importance of respiration in the carbon balance of plants is well known, but its study in grapevine (*Vitis vinifera* L.) is still scarce. Therefore, there is a need to accurately estimate the carbon balance of crops, as well as the effect of genotype, environmental conditions and agronomic practices. Due to the difficulties to accurately measure CO2 exchange in field conditions, the carbon balance in vines has been determined from the accumulation of dry mass per year or from the CO_2 exchange at the whole plant level. However, few studies integrate the respiratory losses of each plant organs to estimate the plant carbon balance. In addition, climate change has an effect on phenology and growth and supposes an increase in respiratory activity of the plant due to the increase in temperature. For this reason, this thesis was proposed with the objective of determining the respiration losses of different plant organs throughout their phenology, as well as their environmental and genetic variations in realistic growing conditions. Based on these determinations, the carbon balance in grapevine and genotype (Garnacha and Tempranillo) and edafo-climatic variations (irrigation and drought) were calculated. The results showed respiratory losses of the aerial parts (leaves, stems and fruits) around 20-30%, representing between 7-8% and 9-11% of total carbon fixed by photosynthesis in irrigation and Drought, respectively. The root was the organ with the highest respiratory losses in relation to the total fixed by photosynthesis (25-30%), obtaining the highest values of the irrigated plants. Also, the irrigation affected the integrated values of carbon fixation and loss due to the accumulated dry matter. The results showed the importance of the respiratory processes, as well as their variability along the phenology, showing a strong effect of the genotype on the airborne organs respiration. The consequence of these differences in the carbon balance was reflected in differences in the accumulation of biomass, with Tempranillo being the cultivar with the highest vegetative biomass (leaves and stems) and Garnacha greater biomass of the reproductive organs and permanent organs.

RESUM

És coneguda la importància de la respiració en el balanç de carboni de les plantes, però queda molt per aprofundir en el seu estudi en vinya (*Vitis vinifera* L.). Per això, hi ha una necessitat d'estimar amb precisió l'efecte de les condicions ambientals, del genotip i de les pràctiques agronòmiques sobre el balanç de carboni de la vinya. La majoria d'estudis han determinat el balanç de carboni en vinya a partir de la biomassa produïda o de l'intercanvi de CO_2 a nivell de planta sencera. No obstant això, els treballs que tenen en compte les pèrdues respiratòries de cada un dels òrgans són escassos. A més, el canvi climàtic té un efecte sobre la fenologia i el creixement i suposa un increment de l'activitat respiratòria de la planta a causa de l'augment de temperatura. Per això, es va plantejar aquesta Tesi amb l'objectiu de determinar les pèrdues per respiració de diferents òrgans de la planta al llarg de la seva fenologia, així com les seves variacions ambientals i genètiques en condicions realistes de cultiu. Basant-se aquestes determinacions, s'han calculat els balanços de carboni en vinya i les variacions per causes genotípiques (cultivars Garnatxa i Ull de llebre) i edafo-climàtiques (reg i sequera). Els resultats van mostrar pèrdues respiratòries dels òrgans de la part aèria (fulles, tiges i fruits) al voltant del 20-30%, representant entre un 7-8% i un 9-11% de carboni total fixat per la fotosíntesi en reg i sequera, respectivament. L'arrel va ser l'òrgan amb registrar majors pèrdues respiratòries en relació al total fixat per la fotosíntesi (25-30%), obtenint els majors valors les plantes regades. Així mateix, el reg va afectar els valors integrats de fixació i pèrdua de carboni a causa de la major matèria seca acumulada. Els resultats van mostrar la importància dels processos respiratoris, així com la seva variabilitat al llarg de la fenologia, mostrant un fort efecte del genotip en la respiració dels òrgans de la part aèria. La conseqüència d'aquestes diferències en el balanç de carboni es va reflectir en diferències en l'acumulació de biomassa, sent Ull de llebre el conrear amb major biomassa vegetativa (fulles i tiges) i Garnatxa major biomassa dels òrgans reproductors i òrgans permanents.

INTRODUCCIÓN

INTRODUCTION

INTRODUCCIÓN

La respiración de las plantas representa un gasto promedio de carbono del 50% que puede oscilar en función de las condiciones ambientales y el momento del ciclo fenológico, pudiendo llegar a representar hasta el 90% respecto del fijado por la fotosíntesis (Amthor 2000). En estudios sobre respiración en ecosistemas naturales, la respiración de hojas, tallos y raíces representa aproximadamente un 60% del total respirado por la biosfera (Law, BE, Ryan, MG, Anthoni 1999, Janssens et al. 2010, Van der Molen et al. 2011, Biederman et al. 2016), lo que supone que la respiración de las plantas tiene un peso fundamental en los balances globales de CO_2 en el planeta. Sin embargo, pese a la reconocida importancia de la respiración, queda mucho por conocer sobre las variaciones de la respiración inducidas por las condiciones ambientales, la fenología y el genotipo. Esta información básica es necesaria para valorar el peso de los procesos respiratorios en la economía del carbono de las plantas y la representación de la respiración en los modelos de ecosistemas terrestres, como han resaltado recientemente diferentes autores (Atkin et al. 2015, Wullschleger et al. 2015).

Está demostrado el comportamiento dinámico de la actividad respiratoria de las plantas a cambios en las condiciones ambientales y su aclimatación a dichas variaciones a lo largo de los diferentes grupos funcionales (Atkin et al. 2015, Reich et al. 2016). Sin embargo, todavía se siguen utilizando modelos de balance de carbono tomando la respiración como un porcentaje de la producción bruta, o como un parámetro dependiente únicamente de cambios en la temperatura a corto plazo [Q10, factor que expresa el incremento de velocidad de un proceso (como la respiración) al aumentar 10°C la temperatura], sin tener en cuenta otros factores importantes como son: el órgano, las características anatómicas y nutricionales del tejido, estado hídrico, el genotipo o la aclimatación a los factores ambientales. Se sabe que la respiración en plantas varía a lo largo de los diferentes biomas y grupos funcionales, altamente influenciada por características del tejido, tales como el contenido de nitrógeno o el peso específico del órgano (Reich, Walters, Ellsworth, et al. 1998a, Reich, Walters, Tjoelker, et al. 1998, Wright et al. 2006).

Sin embargo, mientras que los estudios enfocados a valorar la importancia de estos procesos respiratorios en ecosistemas naturales son numerosos, los estudios sobre respiración en cultivos son todavía escasos. Es necesario incrementar este conocimiento por la contribución de la agricultura a la reducción del incremento del CO_2, pero

también, porque parte de estos procesos resultan esclarecedores en relación con la productividad de los cultivos y los parámetros de calidad de la cosecha.

La agricultura ocupa un 38.4% de la superficie emergida del planeta, y dentro de Europa supone el uso del suelo más importante, lo que refleja la importancia de la agricultura en el balance global de carbono terrestre (FAO, 2015). El objetivo de la agricultura del siglo XXI es obtener la máxima producción de forma ambientalmente sostenible y siempre teniendo en cuenta los objetivos de calidad deseados por el productor. Dentro de los diferentes criterios de sostenibilidad de la producción agraria, destaca la reducción de la huella de carbono y el papel de los cultivos como secuestradores de carbono. Para su correcta cuantificación, es necesario determinar las pérdidas respiratorias de los diferentes órganos de la planta y su variabilidad a lo largo del ciclo del cultivo, todo ello en función del genotipo, las condiciones ambientales y las técnicas culturales utilizadas.

En el caso concreto del cultivo de la vid, las referencias bibliográficas muestran variaciones muy amplias con una base fisiológica (ciclo de vida), ambiental (temperatura, estrés hídrico) y genética. Los modelos de crecimiento del viñedo se basan en la distribución de biomasa entre los órganos de las plantas a lo largo del ciclo fenológico de las mismas (Wermelinger et al. 1991, Castelan-Estrada et al. 2002, Vivin et al. 2002, Scandellari et al. 2016), y son muy escasos los estudios que han tenido en cuenta el coste respiratorio de los diferentes órganos a lo largo del ciclo fenológico de las plantas (Poni et al. 2006, Escalona et al. 2012a). Por todo ello, para determinar correctamente los balances de carbono, es necesario conocer las variaciones de la respiración en función de factores como el órgano de la planta, la edad del mismo, la fenología, el genotipo y los factores ambientales. Así mismo, el amplio espectro en la procedencia de los datos de respiración hace necesario contrastar medidas en condiciones controladas en maceta, con medidas en condiciones reales de cultivo. Por todo ello, hay una necesidad de generar más información para conocer y valorar mejor la importancia del gasto respiratorio de los diferentes órganos y la contribución de los mismos al balance de carbono y a la productividad de la vid.

1. La respiración: Componentes

La respiración, pese a registrar tasas muy por debajo de la fotosíntesis, supone un gasto mantenido durante el día y la noche y en todos los órganos de la planta, tanto

aéreos como subterráneos. Estas tasas respiratorias son mucho más altas en órganos en crecimiento y pueden descender a valores extraordinariamente bajos en órganos en reposo. Así, en los estudios de respiración de las plantas se han considerado comúnmente dos componentes: respiración de crecimiento y respiración de mantenimiento. La respiración de crecimiento se define como la energía necesaria para convertir los carbohidratos no estructurales en nuevos constituyentes de un determinado órgano, y la respiración de mantenimiento como la energía necesaria para renovar proteínas, mantener el gradiente osmótico de las membranas, así como los procesos metabólicos asociados a ajustes fisiológicos.

2. Variación de la respiración entre los diferentes órganos y a lo largo del ciclo fenológico de la vid

El gasto respiratorio de carbohidratos respecto del total que se producen durante la fotosíntesis depende en gran medida del órgano concreto de la planta (Laureano et al. 2008), debido entre otros factores, al diferente coste energético de construcción de cada uno de ellos (Vivin et al. 2003). La mayoría de valores de respiración en los estudios realizados hasta el momento se refieren a hojas (Schultz 1991, Escalona et al. 1999, 2003, Zufferey et al. 2000, Gómez-Del-Campo et al. 2004, Weyand and Schultz 2006, Zufferey 2016). En este órgano, hay un claro efecto de la edad, así como del contenido de nitrógeno en los valores de respiración (Zufferey 2016). En la fase de expansión de la masa foliar del cultivo, la respiración de crecimiento se ve asociada a importantes incrementos en área foliar, lo que implica altos costes en la expansión de la hoja. La parada de crecimiento vegetativo conlleva una disminución de las tasas de respiración por planta, que en adelante se reducirá a la respiración de mantenimiento hasta el final del ciclo vegetativo (Schultz 1991, Poni et al. 2006). La tasa de respiración del tallo también se ve afectada por el crecimiento del mismo, así como por el proceso de lignificación o agostamiento, registrándose altas tasas de respiración en los estados fenológicos de máximo desarrollo vegetativo (Palliotti and Cartechini 2005, Poni et al. 2006) y disminuyendo hasta registrar una tasa de respiración de mantenimiento sostenida hasta el final del ciclo fenológico.

Todos los órganos aéreos de la vid contienen clorofila, indicando la posibilidad de actividad fotosintética en todos ellos (Zamski and Schaffer 1996). Por ello, aparte de la ampliamente estudiada fotosíntesis en hoja, se han determinado tasas fotosintéticas en tallos jóvenes y maduros, así como en zarcillos (Kriedemann 1968, Palliotti and

Cartechini 2001, 2005), que, aunque sean bajas en comparación a la hoja, contribuyen al balance de carbono de la planta. Así los tallos jóvenes son capaces de reducir su coste respiratorio en un 70% bajo el efecto de la luz difusa y en un 89% bajo el efecto de radiación solar reflejada (Kriedemann 1968), lo que constituye otro factor a tener en cuenta en el manejo de la intercepción de luz en el viñedo.

La respiración del fruto y de las raíces constituyen los componentes más importantes dentro de la respiración total de la planta (Ollat and Gaudillere 1996). La respiración del fruto ha sido estudiada desde el estado de floración (Palliotti and Cartechini 2001) hasta la maduración (Poni et al. 2006), pasando por los estados intermedios de crecimiento (Ollat and Gaudillère 2000) en plantas en maceta y condiciones controladas. La contribución de la respiración de racimo a la respiración total de la planta varía a lo largo del desarrollo de las bayas, llegando a registrar los valores más altos de respiración cuando la baya tiene las tasas de crecimiento relativo más altas, al igual que el resto de órganos en la planta. La capacidad fotosintética del fruto se ha relacionado, al igual que las hojas, con el contenido de clorofila y la densidad de estomas. La degradación de la clorofila a lo largo del desarrollo de la baya hace que la capacidad de fijación de CO_2 de las bayas dependa del estado de crecimiento en el que se encuentren (Ollat and Gaudillère 2000, Palliotti and Cartechini 2001, Poni et al. 2006, Dai et al. 2010). Así mismo, la diferente concentración en clorofila entre las partes que componen la baya (exocarpo, mesocarpo y semillas), hacen que cada una de ellas tenga una contribución específica en el metabolismo y en el balance de carbono del fruto (Breia et al. 2013).

Tal como se detalla anteriormente, otro órgano de gran importancia en el balance de carbono de la vid es la raíz. La respiración de raíz genera la energía necesaria para crecer y para mantener el sistema radicular, así como para la absorción de iones y su transporte hacia el xilema. De acuerdo con Lambers et al., (1996) la actividad respiratoria de la raíz consume entre un 10-50% de carbono, respecto del total fijado por la fotosíntesis. En viña, pese a la importancia del componente radicular en el balance de carbono, los estudios son todavía escasos (Morinaga et al. 2003, Comas et al. 2005, 2010, Huang et al. 2005, Franck et al. 2011a, Escalona et al. 2012a) debido a las dificultades técnicas que implica su medida. El patrón de la respiración de raíz está influenciado por el estado fenológico de la planta, así como por el contenido de agua en el suelo que, sobre todo en condiciones de sequía, varía a lo largo del ciclo vegetativo y

reproductivo. De ahí la importancia de su estudio para tener un mejor conocimiento de su dinámica a lo largo del ciclo vegetativo de la planta. La variabilidad en la respiración de la raíz en el cultivo de la vid es un tema a explorar en la actualidad, ya que está sujeta a multitud de variables como son la diversidad de portainjertos utilizados y su interacción con los cultivares de *Vitis vinifera*, diversidad de tipos de suelo, así como técnicas de manejo que se utilizan en el viñedo (Ollat et al., 2016). El conocimiento y análisis de esta variabilidad (genética, ambiental, estacional y espacial) en el componente respiratorio de los balances de carbono puede llevar a mejorar en un futuro cercano la toma de decisiones a la hora de implantar un nuevo viñedo o a la hora de decidir ejecutar una determinada técnica de manejo.

3. Variaciones de la respiración por el efecto del genotipo, de las condiciones ambientales y nutricionales de la vid.

Aparte de la variabilidad en las tasas respiratorias entre diferentes órganos, y las diferencias registradas durante las distintas fases de crecimiento de los mismos, las condiciones ambientales y el estado nutricional son otros de los factores que van a condicionar las tasas respiratorias. La mayoría de los estudios en vid que se refieren al estudio de los factores ambientales sobre la respiración se basan en el estudio del efecto de la temperatura a corto plazo (Schultz 1991, Zufferey et al. 2000, Poni et al. 2006, Weyand and Schultz 2006, Escalona et al. 2012a, Zufferey 2016) . De esta manera, las pérdidas respiratorias en la planta se calculan utilizando un modelo de respiración empírico (Schultz 1991), basado en la relación exponencial de la respiración nocturna respecto de la temperatura en varios puntos a lo largo de la fenología. Esta respuesta exponencial a la temperatura (Q10) se ha demostrado en todos los órganos aéreos jóvenes (hojas, tallos y frutos), es decir en órganos en crecimiento. Cuando los órganos completan su madurez, el efecto de la temperatura a corto plazo sobre la respiración de los tejidos se reduce notablemente (Poni et al. 2006).

En cuanto al efecto genético, la respiración de órganos aéreos se ve afectada por la especie (Galmés et al. 2007), y dentro una misma especie, por el genotipo (Lambers et al. 2008). Generalmente, las plantas denominadas “de crecimiento rápido” se asocian con costes respiratorios de crecimiento mayores. Esta misma visión se podría trasladar al diferente patrón de crecimiento entre genotipos de una misma especie. En el cultivo de la vid se encuentra una gran variedad de patrones de crecimiento, así como de estructura de hoja (tamaño, grosor y densidad del tejido), lo cual puede condicionar las

tasas respiratorias de crecimiento y mantenimiento. En hoja, algunos trabajos que comparan diferentes cultivares de vid no demostraron diferencias significativas en las tasas respiratorias (Zufferey et al. 2000, Gómez-Del-Campo et al. 2004). Sin embargo, las diferencias en la estructura de hoja, así como otras características observadas para diferentes cultivares de vid (Tomás et al. 2014, Martorell et al. 2015a), sugieren que hay cierta variabilidad genotípica de la respiración durante la expansión de hoja en condiciones de campo. En el fruto, la diversidad genética en relación a su capacidad productiva, así como en las características del fruto como pueden ser la forma, tamaño, compacidad del racimo o la ratio pulpa/hollejo, determinan muchos de los parámetros de calidad en el mosto. Esta diversidad puede asociarse con cambios sustanciales en el coste de construcción de las bayas. Se sabe que las semillas tienen un coste de carbono más elevado que otros tejidos del fruto. Todo ello hace interesante el estudio y la comparación entre diferentes genotipos con el objetivo de investigar las oportunidades de mejora genética para favorecer el balance de carbono de la planta y del fruto en particular.

Así como el factor genético juega un papel importante en los órganos de la parte aérea, no se conoce el efecto del genotipo sobre los costes respiratorios en la raíz. Hasta el momento, se desconoce si la respiración radicular de un mismo portainjerto se ve condicionada por las diferencias en la parte aérea de los diferentes cultivares injertados. Por ello, es necesaria más información acerca del efecto genético (relativo a los diferentes portainertos utilizados en viticultura) sobre la respiración de raíz.

A su vez, la demanda respiratoria depende de la composición química del tejido (Amthor 1989), en mayor medida el contenido de nitrógeno y de azúcares disponibles. A pesar de que hay numerosos estudios que muestran la importancia del contenido de nitrógeno de los tejidos a la hora de modelizar la respiración (Reich, Walters, Ellsworth, et al. 1998b, Laureano et al. 2013), hay muy pocos trabajos que muestren la relación de estos dos parámetros en vid. Por tanto, el estudio de la evolución de la respiración asociada al contenido de nutrientes en el tejido, fundamentalmente el papel del Nitrógeno en este proceso, puede aportar información sobre la eficiencia en el uso de recursos en la planta, así como las posibilidades de explorar mejoras genéticas en este campo.

4. Efecto de las técnicas agronómicas sobre la respiración de los diferentes órganos.

Los efectos de las prácticas culturales del viñedo sobre los balances de carbono han sido analizados por diferentes autores (Lakso and Wünsche 1999, Poni et al. 2000, 2006, Palliotti et al. 2004, Lakso et al. 2008, Tarara et al. 2011). La mayor parte de estos estudios se centran en el efecto sobre la fijación de carbono, pero no en la variabilidad de la respiración. Por ejemplo, son numerosos los estudios que analizan los efectos del estado hídrico de la planta sobre la capacidad fotosintética de las hojas (Escalona et al. 1999, 2003, Flexas and Medrano 2002, Medrano et al. 2003, Collins et al. 2010, Chaves et al. 2010). Sin embargo, el análisis de los efectos a corto y largo plazo sobre la respiración están siendo estudiados en la actualidad (Van der Molen et al. 2011), y sigue creando controversia, pudiendo aumentar o disminuir, dependiendo del tipo de planta y su tamaño o edad, llegando incluso a aumentar como respuesta al déficit hídrico (Flexas et al. 2006). Al-Hazmi et al. (1996) encontró una pequeña disminución de la respiración en oscuridad en plantas en maceta de Cabernet Sauvignon bajo un tratamiento de estrés hídrico. Sin embargo, Salazar-Parra et al. (2015a) no detectaron ninguna diferencia entre plantas de Tempranillo bien regadas y parcialmente regadas.

Las diferentes estrategias de riego deficitario como el riego deficitario controlado (Romero et al. 2014, Salazar-Parra et al. 2015b) o el riego parcial de raíces han sido estudiadas para conocer las consecuencias fisiológicas de su práctica (Pagay 2016). Sin embargo, los efectos sobre los gastos respiratorios no se conocen hasta la fecha. Todo ello sugiere que son necesarios más estudios sobre el efecto de la sequía en diferentes condiciones agronómicas, y su contribución al balance de carbono de la planta.

Otro aspecto agronómico que puede modificar sustancialmente los diferentes componentes del balance de carbono del viñedo es el sistema de conducción y poda utilizados, ya que determinan la disposición del dosel de la planta y por tanto la captación de la radiación por las hojas a lo largo del día. En relación a la respiración foliar, Lakso and Wünsche (1999) encontró que la respiración en oscuridad en sistemas de conducción de poda mínima fue mayor a principios del ciclo vegetativo como respuesta a la aceleración del crecimiento de la vegetación es este tipo de formación. Sin embargo, Poni et al. (2000) mostraron valores de respiración similares para los sistemas de conducción de poda mínima y espaldera vertical. Así mismo, en esta línea, Weyand and Schultz (2006) basaron las pérdidas respiratorias en la asunción de que no hay diferencias en respiración entre sistemas de conducción. Todo ello apunta a que los

modelos de cultivo desarrollados hasta la fecha requieren una mayor documentación sobre respiración de los diferentes órganos de la vid y los factores que lo regulen, que ayuden a entender y estimar de forma precisa las consecuencias de implementar ciertas técnicas de manejo en el viñedo.

5. Respiración y balances de carbono en vid: Integración de procesos en condiciones de campo

En la actualidad, se han llevado a cabo diferentes técnicas y aproximaciones para estimar el balance de carbono neto de la planta con el objetivo de estimar el carbono que es capaz de secuestrar el viñedo. Una de las técnicas más extendidas es la medida de la biomasa producida y su evolución a lo largo del ciclo fenológico de la planta (Greer and Sicard 2009, Greer et al. 2011, Greer 2017), lo que ha permitido desarrollar modelos de distribución de carbono en vid partiendo de entradas sencillas en el modelo, como son las condiciones ambientales o parámetros agronómicos del histórico registrado en un viñedo (Lakso and Wünsche 1999, Poni et al. 2006, Lakso et al. 2008). La medida destructiva de biomasa implica pesar los diferentes órganos de la planta, lo que supone una información valiosa, pero a la vez, costosa a la hora de hacer medidas a gran escala. Esta limitación ha derivado en el desarrollo de modelos alométricos que relacionan las medidas directas de biomasa con otras medidas más sencillas no destructivas y con otros parámetros medidos en el viñedo (Vivin et al. 2002, Miranda et al. 2017). Sin embargo, todas estas técnicas no nos dan información detallada sobre la dinámica de los flujos de entrada y salida de CO_2 que se producen a lo largo del ciclo fenológico de la planta en condiciones de campo. El intercambio de CO_2 en vid se ha determinado utilizando diferentes modalidades de cámara de planta entera (Tarara et al. 2011, Poni et al. 2014, Escalona et al. 2016), con la que se obtiene un flujo de CO_2 neto pero solamente de la parte aérea de la planta. Además, aunque las medidas con cámaras de planta entera reflejan el flujo neto de CO_2 que proviene de la parte aérea, no nos dan información sobre el peso de cada uno de los órganos. Para determinar la contribución particular de cada órgano, es necesario cuantificar la fotosíntesis y la respiración de los diferentes órganos de la planta a lo largo de su ciclo fenológico teniendo en cuenta los distingos factores que afectan a su variabilidad.

Como se ha detallado en los epígrafes anteriores, estos procesos fisiológicos dependen de numerosos factores como son las condiciones ambientales, la fenología o las prácticas agronómicas. Por ello, para llevar a cabo una integración a nivel de planta

entera en condiciones de campo, es necesario considerar la contribución de cada órgano teniendo en cuenta diferentes edades y exposiciones a la luz, orientaciones, posiciones dentro del sarmiento y el estado hídrico y nutricional de la planta. Todas estas variables afectan a la respiración y al balance de carbono de diferente forma, y su estudio es todavía escaso en el cultivo de la vid. Por ello, es necesaria más información en este ámbito con el fin de crear modelos de cultivo que integren de una forma precisa los aspectos agronómicos y fisiológicos de la planta, que ayuden a un manejo efectivo del viñedo y a un uso eficiente de los recursos.

OBJETIVOS

OBJECTIVES

OBJETIVOS

Los antecedentes reseñados en la Introducción muestran la importancia de los procesos respiratorios y, a la vez, la falta de información precisa sobre las variaciones de estos procesos particularmente en condiciones realistas de cultivo.

El análisis de estos antecedentes es la base del planteamiento de esta tesis cuyo objetivo general es aportar nueva información sobre las variaciones en las tasas respiratorias de los diferentes órganos a lo largo del ciclo fenológico, en diferentes condiciones ambientales y en dos genotipos de vid. La hipótesis de trabajo es que dichas variaciones genotípicas y ambientales afectan al componente respiratorio de los balances de carbono en condiciones reales de cultivo. Esto tiene gran importancia para conocer mejor la fisiología de la planta en condiciones de campo y a su vez, esta información puede ser útil para mejorar las técnicas agronómicas enfocadas a una viticultura sostenible.

En este sentido se plantean a continuación los objetivos generales y específicos de la presente Tesis.

- El objetivo general fue determinar las pérdidas por respiración a lo largo de la fenología, así como sus variaciones ambientales y genéticas en condiciones realistas de cultivo; y en base a estas determinaciones, valorar los balances de carbono en vid y las variaciones genotípicas y edafo-climáticas.

Los objetivos específicos, están asociados a cada uno de los 4 capítulos (publicaciones) que componen el apartado de resultados de la presente Tesis:

- Estudiar las tasas de respiración asociadas a hojas en expansión y totalmente adultas de los cultivares Garnacha y Tempranillo en condiciones de campo, y analizar la relación de la respiración con características morfológicas y químicas de la hoja de ambos cultivares (Capítulo 1).
- Determinar la importancia de las prácticas de manejo sobre la respiración de fruto en condiciones de campo, e investigar el efecto del cultivar y del estado hídrico de la planta sobre las tasas respiratorias y fotosintéticas del fruto (Capítulo 2).
- Examinar la variabilidad espacial y estacional de la respiración de suelo asociada al ciclo fenológico de la planta y al estado hídrico de la misma, así como

estudiar el componente de respiración asociado a la raíz y a la respiración basal del suelo (Capítulo 3).

- Determinar el efecto del genotipo y el estado hídrico de la planta sobre los costes respiratorios integrados anuales de cada uno de los órganos y sobre el balance de carbono de la planta en condiciones reales de campo (Capítulo 4).

CAPÍTULO 1

CHAPTER 1

Leaf growth rate and nitrogen content determine respiratory costs during leaf expansion in grapevines

Leaf growth rate and nitrogen content determine respiratory costs during leaf expansion in grapevines

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Running title: Leaf characteristics determine R_d during leaf expansion

Abstract

The relationship between leaf expansion process and leaf respiration rates is well known along different species, but the intraspecific variability of that relationship has received little attention. Therefore, night respiration (R_n) and its associated growth (R_g) and maintenance (R_m) components were evaluated during leaf expansion in two reputed grapevine cultivars (Tempranillo cv. And Grenache cv.) that differ in their plant growth pattern. Simultaneously, leaf traits as leaf mass area, nitrogen and carbon content were evaluated in order to relate to respiratory processes and leaf growth.

The first expanded leaves from the shoot apex were labeled during an active shoot growth period (at the beginning of flowering stage). Leaf respiration, R_n (R_g and R_m), single leaf area (LA), dry weight (DW), leaf mass area (LMA), nitrogen (N) and carbon (C) content were measured in the labeled leaves until the leaf expansion was completed during 2013 and 2014 seasons. Simultaneously, mature leaves from the same shoots were measured in order to know the R_n rates in fully-expanded leaves.

The results showed differences in the leaf expansion pattern and the associated respiration rates. Tempranillo cv developed leaves with higher LA and lower DW per leaf unit than Grenache cv. Although differences between cultivars were observed in

terms of growth costs in expanding leaves, the maintenance costs were similar for Grenache and Tempranillo. Also, a significant linear regression was found between respiration rates and N content in expanding and mature leaves.

The results indicate that differences in structure and nitrogen content of expanding leaves may lead to respiratory differences between cultivars. These results also demonstrate the importance of respiratory cost components in carbon balance calculations in grapevines.

Introduction

It is well known the importance of plant respiration in the ecosystem, as plant respiration releases around 6-8 times more CO_2 into the atmosphere than the fossil fuels combustion, and leaf respiration represents half of the CO_2 emissions from the plant respiration (Atkin et al. 2014). Agricultural land covers 38.4% of the terrestrial land area (FAOSTAT, 2015); it is the most relevant land use in Europe, and in Spain, agriculture accounts for almost half of the total land surface (INE 2015). Therefore, the study of respiration in crops is important to calculate carbon balances accurately. In grapevines, (Palliotti et al. 2004, Palliotti and Cartechini 2005) showed how most of the aerial plant respiration losses were due to leaf respiration, and the results obtained by (Escalona et al. 2012b) showed that leaf respiration represented around 10% of the carbon fixed by photosynthesis in an experiment with potted vines.

Respiration provides the carbon skeletons and energy needed for tissue growth and maintenance processes (Bouma 2005). The relationship between night respiration (Rn) and leaf traits (e.g. leaf structure and nitrogen content) was demonstrated by different authors (Reich, Walters, Ellsworth, et al. 1998a, Galmés et al. 2011, Laureano et al. 2013, Atkin et al. 2015). Although many studies have examined the interspecific relationship among R_n, leaf expansion and other leaf traits (i.e.: leaf morphology, N content, net photosynthetic capacity), more information is needed in crops, and particularly in grapevines, where the leaf growth pattern greatly differs among cultivars.

Leaf respiration can be partitioned into growth and maintenance components. Growth respiration (R_g) can be defined as the respiratory energy required to convert non-structural carbohydrates into new leaf constituents, and the maintenance respiration (R_m) as the respiratory energy associated with the energy-consuming processes that maintain cellular structure (Bouma 2005, Florez-Sarasa et al. 2007, Lambers et al.

2008). From the different methods used to estimate these respiratory components (Lambers and Ribas-Carbó 2005, Lambers et al. 2008), the regression approach is one of the methods extensively used to estimate respiratory costs of growth and maintenance. These methods require plotting respiration against relative growth rate (RGR). The slopes of the regression lines represent the specific costs for growth, and the regression line intercepts are used to estimate specific costs for maintenance (Lambers 2008)).

It is well known that differences in growth and maintenance costs, may be important to explain differences in growth rates (Lambers et al. 2008, Laureano et al. 2013). In grapevines, few studies were found showing R_n rates in order to compare different cultivars in potted vines (Gómez-Del-Campo et al. 2004, Escalona et al. 2012b), or under field conditions (Schultz 1991, Zufferey 2016). Others authors studied the interspecific variations in RGR or leaf morphology in grapevines (Tomás et al. 2014). However, the R_g and R_m pattern and leaf traits associated during the process of leaf expansion, comparing different grapevine cultivars under realistic conditions has not been described in any of those studies. Therefore, it is necessary to quantify and qualify the cost of production and maintenance of leaves considering the intraspecific variability of leaf traits and structure in order to have an exhaustive knowledge and an accurate carbon balance calculation among different cultivars.

In consequence, the main objectives of this work were: i) to study the leaf expansion pattern and the structural differences between cultivars; ii) to analyze R_n from expanding and mature leaves and the growth and maintenance costs associated, and finally, iii) to relate R_n to leaf expansion, morphological characteristics and N content of leaves from vines under field conditions.

Materials and methods

Plant material and treatments

This study was conducted in the experimental vineyard of the University of Balearic Islands (Palma, 39°38′17″N 2°38′54″E) during two consecutive years (2013 and 2014) using two *Vitis vinifera* grapevine cultivars widely cropped in Spain: Grenache and Tempranillo. Vines were planted in 2009 in rows (distance between rows was 2.5 m and between plants 1 m) and grafted onto rootstock 110 Richter. Vines were trained to bilateral cordons and spur pruned with an average of 12 buds per vine. Soil

type was a typical clay-loam, maintained free of weeds by surface tillage to facilitate measurement of soil respiration.

For this study, the first expanded leaves from the apex of each shoot (Figure 1) were labeled in four plants per cultivar and treatment at the beginning of flowering stage (4th of May 2013 and 12nd of May 2014, respectively), in order to preserve labeled leaves from the destructive measurements of LMA and N-C content. Next day to the leaves were labeled, the experiment was carried out across 31 days, in order to follow the expansion of the labeled leaves in a period of active growth in vines. Simultaneously, a mature leaf from the same shoot was measured in order to study leaves that had completed the expansion period.

Figure 1. View of a shoot apex on 24th of May 2013 in a Grenache vine. A red label was set in the first expanding leaf of each shoot apex from the vines under control (around 12 shoots per vine) on 12nd of May 2013 and on 4th of May 2014. The leaf with the red label in the picture represents one of the studied leaf in the experiment from 2013, 13 days after labeling the leaves.

Measurements were carried out on the nights 1, 3, 7, 13, 18 and 30 after labeling the leaves from the shoot apex. R_n was measured between 11 pm and 1 am, on expanding (labeled) and mature leaves, using a portable gas-exchange analyzer (Li-6400, Li-Cor Inc., Lincoln, NE). Measurements were taken until a stable respiration rate was reached. Immediately after R_n measurements, each leaf was collected and a picture was taken in order to measure the leaf area (LA) using the Image J ® software. Each leaf was dried (80 ºC) and weight, and LMA was calculated from the division of LA into dry weight (DW) of each measured leaf. During 2014, LMA was calculated from two leaf discs from the sampled leaves, dried and weighed (80ºC) in order to obtain the

evolution of LMA of each measured leaf. Also, during 2014 one leaf disc was sampled and dried from the same leaves in order to measure the total N and C content per dry mass. The measurements of N and C content were based on the Dumas method, using an infrared analyzer for C determination, and a thermal conductivity analyzer for N determination (TruSpec CN Leco, Michigan).

In order to estimate the growth and maintenance components of R_n in expanding leaves, a regression approach was carried out between the specific growth rate (SGR) and the R_n (Lambers and Ribas-Carbó 2005). The leaf mass increase from each expansion period was used to calculate the specific growth rate (SGR) as the difference in mass divided by days of growth. The R_n for each leaf was expressed per dry weight unit. A linear regression of R_n was performed against SGR for each cultivar. The slope (mg CO_2 g^{-1}) represents respiration associated mainly with tissue synthesis (growth respiration), while the Y intercept (mg CO_2 g^{-1} day^{-1}) represents the respiration rate at zero growth, i.e. respiration associated mainly with tissue maintenance (Lambers and Ribas-Carbó 2005).

Statistics

Data was processed using analysis of variance (ANOVA) procedures, and means were separated by Tukey´s Test. The data were analyzed using JMP® 12.2.0 (SAS Institute Inc., Cary, NC, USA). For the estimation of R_g and R_m, R_n was regressed (linear model) against SGR. For the detection of differences in R_g and R_m, the slopes and intercepts of the regression lines for R_n vs. SGR were subjected to an ANCOVA analysis.

Results

Leaf expansion pattern

The kinetics of growth and the structure of young leaves markedly changed from Grenache to Tempranillo. Figure 2 shows the growth pattern of expanding Grenache and Tempranillo leaves, expressed as the evolution of LA and DW per leaf unit. The LA and DW followed a logarithm function; however, the relationship between thermal time (cumulated degree day) and LA-DW was linear in the first phase of the leaf expansion (20 days) after the leaves were labeled (Figure 2A and Figure 2B; r^2=0.99 and r^2=0.99, respectively). Figure 2C shows the relationship between thermal time and LMA,

following a linear function. An ANCOVA analyses was made in order to analyze the slopes of the linear regressions from Grenache and Tempranillo (Figure 2A, 2B, 2C). Differences in slope were found between both cultivars in terms of LA and DW, reflecting that the growth rates of Tempranillo were greater than the Grenache ones during the first phase of the leaf expansion. On the other hand, in terms of LMA, no differences in slope were found between cultivars, despite the fact that Grenache displayed greater LMA in every sample date. After 20 days of expansion, the LA, DW of leaf unit and LMA increase slowdown to rich the maximum when the leaf maturation was completed. Final LA largely differed between cultivars, and Tempranillo registered the highest LA values and the fastest leaf expansion, from the first stages of the leaf expansion until the leaf expansion was completed. Final LA of a fully expanded leaf varied from around 200 cm^2 for Grenache to around 300 cm^2 for Tempranillo.

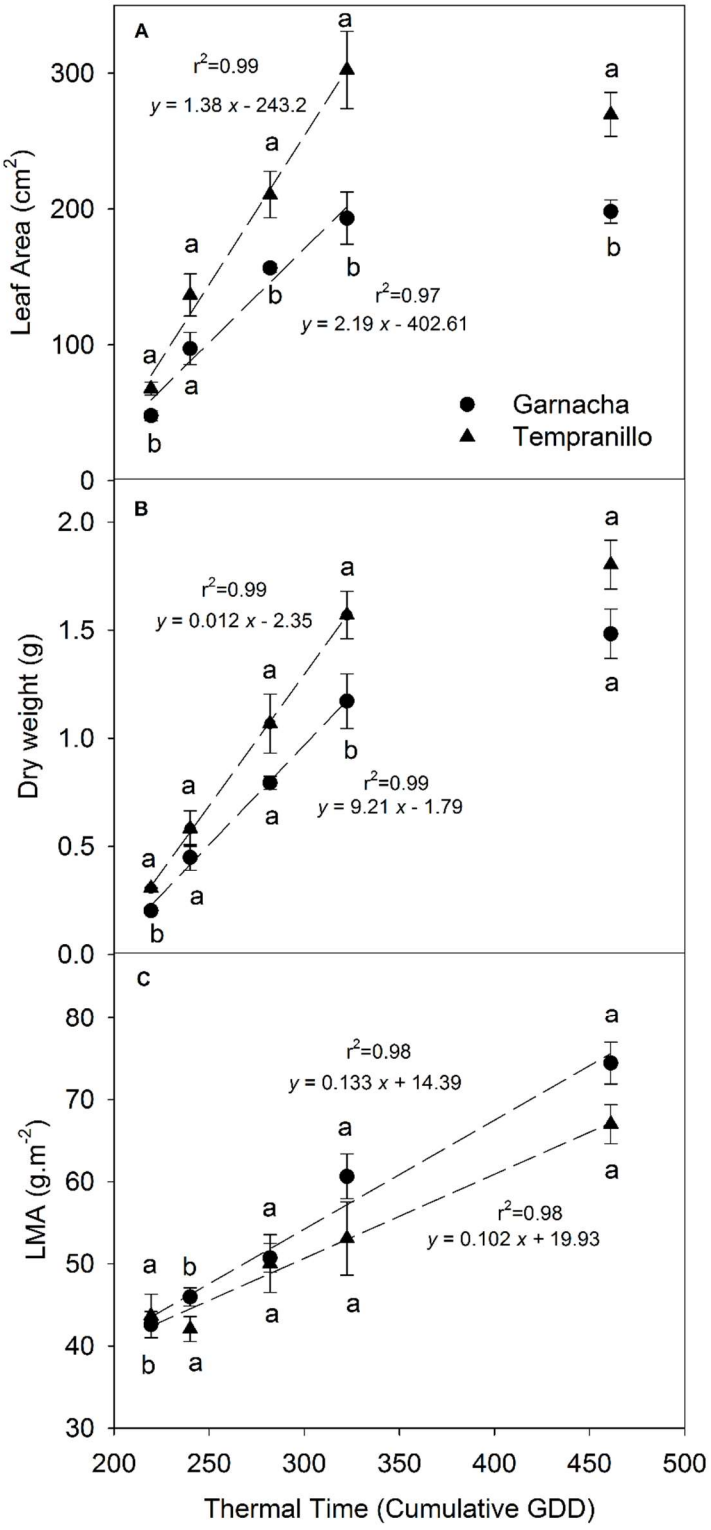

Figure 2. Changes on (A) leaf area (LA), (B) dry weight (DW) and (C) leaf mass area (LMA) along leaf expansion of Grenache (circles) and Tempranillo (triangles) expanding leaves in 2013, from the leaves were unfolded until the leaf expansion was completed. Bars indicate ± SE (n=6).

Night respiration rates during leaf expansion

The R_n pattern of expanding and mature leaves during the flowering stage of 2013 and 2014 is shown in Figure 3. The evolution of R_n was studied from the earliest development stages of each labeled leaf (Figure 1), and throughout the leaf growth, until the leaf expansion was completed. Also, R_n of a fully expanded and mature leaf was measured at the same time to study the R_m. For the expanding leaves (Figure 3A and Figure 3C), the trend of R_n was the same in both cultivars and seasons. However, significant differences between cultivars were found in the first stages of the leaf growth in 2013 and 2014 seasons, and the expanding leaves of Tempranillo registered higher values of R_n expressed by dry weight unit (0.035-0.04 $\mu molCO_2\ g^{-1}\ s^{-1}$) than Grenache (0.025-0.035 $\mu molCO_2\ g^{-1}\ s^{-1}$). R_n per dry weight unit was significantly higher in growing leaves than in mature leaves during the first days of its expansion. After this period, respiration rates were similar in both types of leaves. Moreover, for the mature leaves, (Figure 3B, Figure 3D) no significant differences between cultivars were observed, maintaining R_m rate around 0.01 $\mu molCO_2\ g^{-1}\ s^{-1}$ all along the experiment.

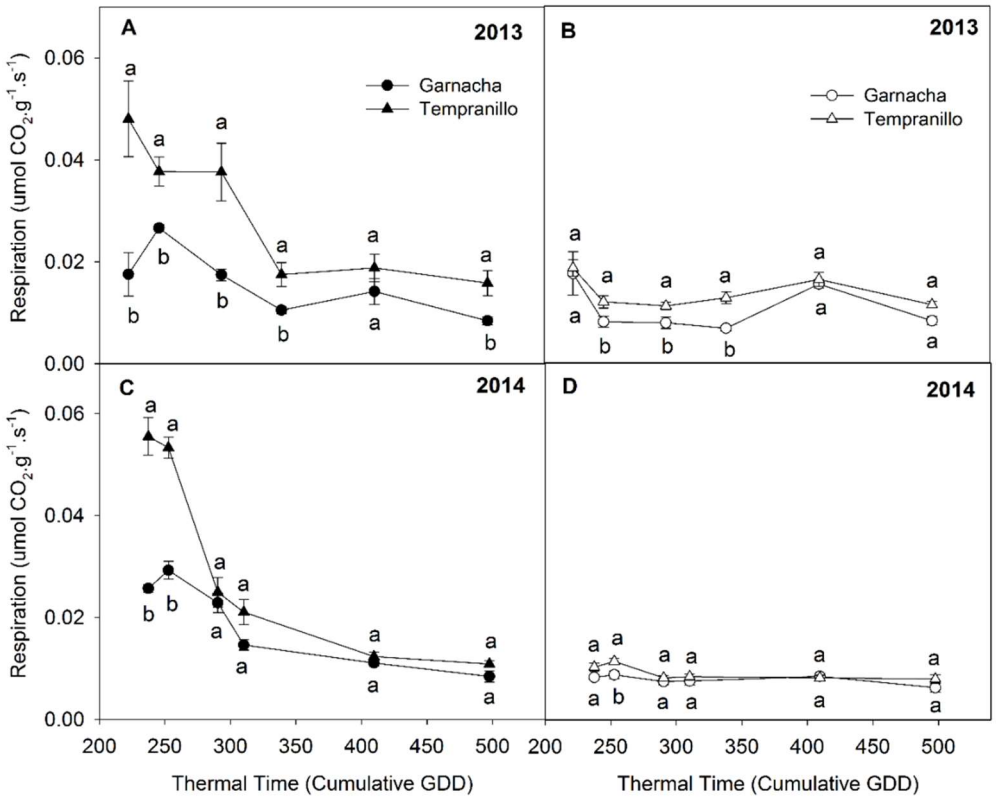

Figure 3. Leaf respiration averages of growing (closed symbols) and mature (open symbols) leaves during leaf expansion in cv. Grenache (circles) and Tempranillo (triangles) vines in 2013 (A and B) and 2014 (C and D) seasons. Bars indicate ± SE (n=6-8). Different letters denote significant differences between cultivars.

Plant growth is associated to respiration, but this relationship varies among species and cultivars. In Figure 4, a significant positive correlation was found in young Tempranillo (r^2 = 0.79, P<0.0005) and Grenache (r^2 = 0.72, P<0.0005) leaves between the SGR and the R_n. Separate slopes analyses were made to test whether the slopes of the linear functions varied between cultivars. Based on this ANCOVA analysis, it was found that there were significant differences in slope (R_g) between both cultivars; thus, R_g per unit mass in Tempranillo expanding leaves was greater than in Grenache. Also, R_m of expanding leaves was estimated as the intercept of the regression lines (Figure 4). R_m was significantly higher (P<0.05) in Grenache growing leaves (R_m = 9.25 $nmolCO_2.g^{-1}.s^{-1}$) than in Tempranillo ones (R_m = 6.24 $nmolCO_2.g^{-1}.s^{-1}$).

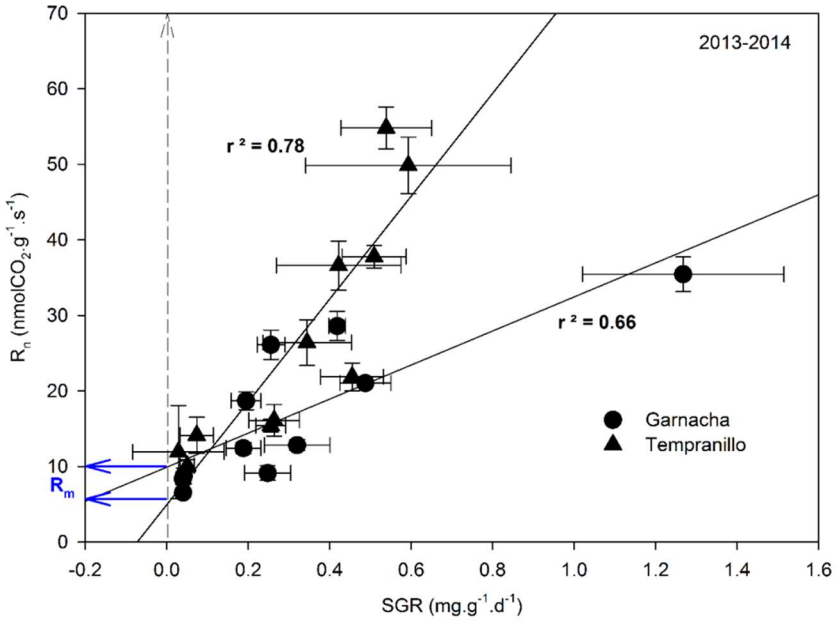

Figure 4. Relationship between the specific respiration rate and night leaf respiration per dry weight (means ± SE, n=6-8) for expanding leaves in Grenache (circles) and Tempranillo (triangles) cultivars. The intercept of each line regressions represents the maintenance respiration (Rm).

Leaf N and C content. Effect on night respiration.

The leaf N and C content was measured in order to study the differences between cultivars. The evolution of the relationship between N-C content and R_n in expanding and mature leaves is showed in Figure 5. The Tempranillo expanded leaves showed a significantly higher (P < 0.05, 0.046 gC. g_{DW}^{-1}) N concentration than the Grenache ones at the first stages of expansion, from where it began to descend gradually to equal the N concentration of the mature leaves. As for the total C content per dry

weight, no significant differences were found between growing and mature leaves, nor between cultivars, maintaining around 0.48 gC. g_{DW}^{-1} all along the experiment.

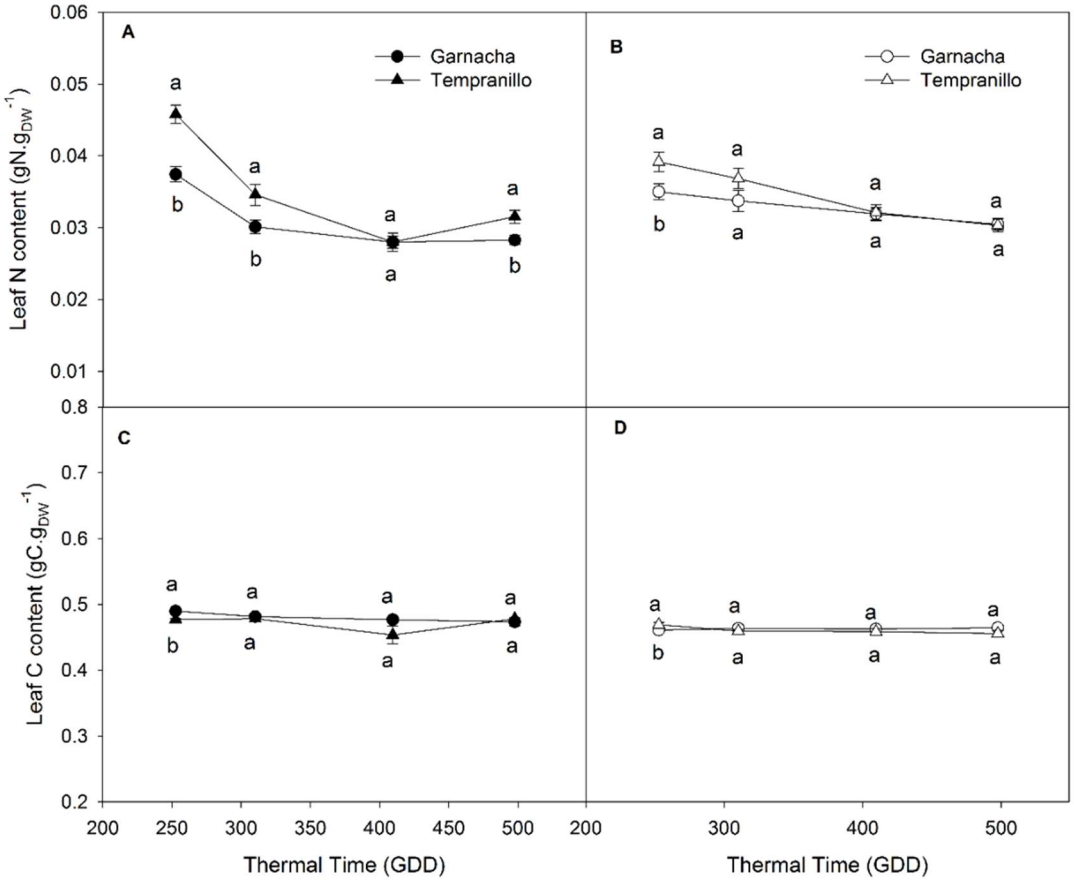

Figure 5. Mean values (± SE, n=8) of nitrogen (A and B) and carbon (C and D) content per dry weight unit in growing (A and C; closed symbols) and mature (B and D; open symbols) leaves for Grenache (circles) and Tempranillo (triangles) during the leaf expansion experiment of 2014. Different letters represent significant differences from ANOVA analysis, $p<0.05$.

In order to study the relationship between the leaf N content and R_n, a correlation between both factors was carried out (Figure 6). Significant linear regressions were found for both mature (R^2=0.44) and growing (R^2=0.7) leaves. The slope of the expanding leaves regression was significantly different from the mature one. Thus, mass-based R_n showed a greater dependence on the N content in the expanding leaves than in the mature leaves.

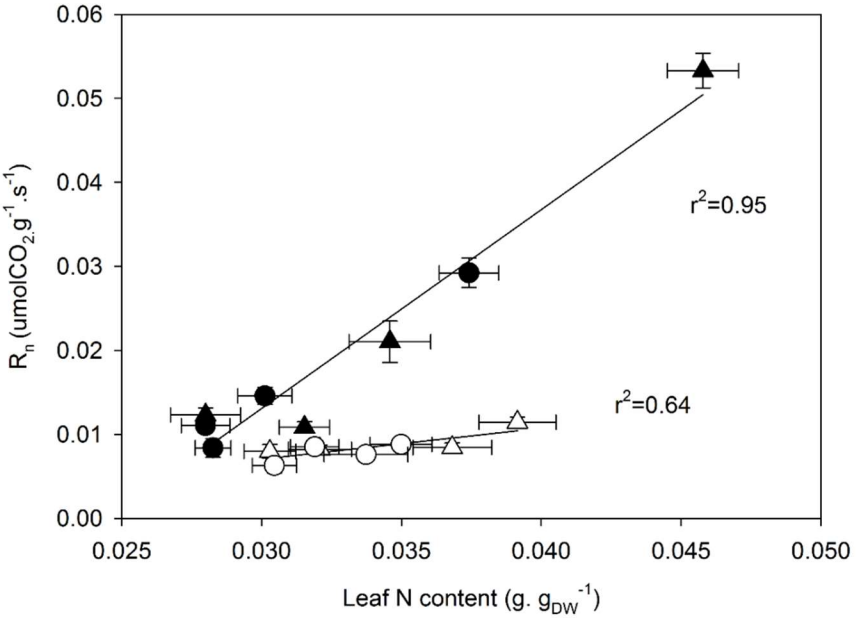

Figure 6. Relationship between leaf nitrogen content per dry weight and night leaf respiration per dry weight (means ± SE, n=8) for expanding (closed symbols) and mature (open symbols) leaves, in Grenache (circles) and Tempranillo (triangles) vines.

The respiration rate per unit of N or C gives us information about N or C use efficiency during the leaf growth. In this sense, the leaves in the first stages of expansion in Tempranillo recorded greater R_n rates per unit of N and C than Grenache (Figure 7A and 7C). However, in mature leaves, no significant differences were found between both cultivars.

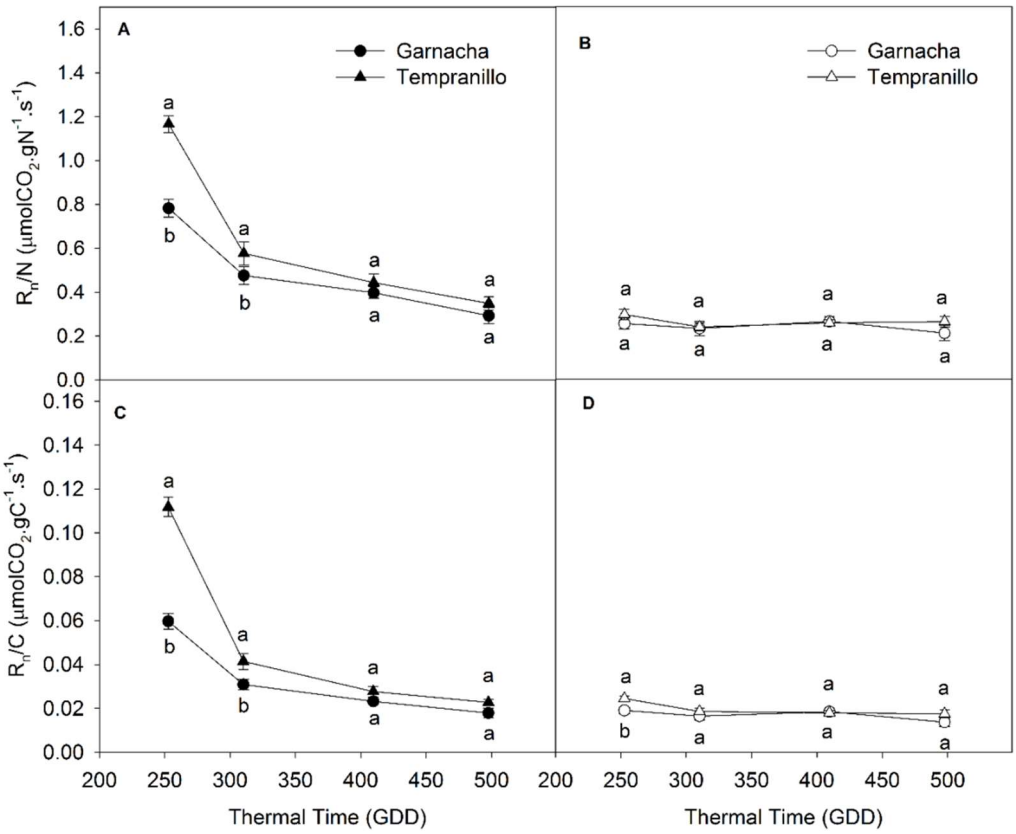

Figure 7. Mean values (± SE, n=8) of R_n per N unit and C unit of expanding (A and C; closed symbols) and mature (B and D; open symbols) leaves in cv. Grenache (circles) and Tempranillo (triangles). Different letters represent significant differences between cultivars from ANOVA analysis, $p<0.05$.

Discussion

Respiration is an important plant process to be taken into account when calculating carbon balances in plants (Valentini 2000). Nowadays, the interest of increasing the knowledge on respiration processes in crops is growing because of a recognized paucity of data on respiration rates and the relevance of this process to understand different physiological responses, such as the whole plant carbon balance. Therefore, the study of grapevine respiratory activity to better understand the genetic and environmental effects is gaining interest and importance (Franck et al. 2011, Zufferey 2016, Hernández-Montes et al. 2017). Inside this frame work, this study analyses the respiration rate activity during the leaf expansion in order to evaluate the growth and maintenance components of grapevine leaves. Leaf area is an important part of the grapevine vegetative growth and results in a development of 2-6 m^2 of new leaves per plant during the first weeks of the vines growth cycle (Gómez-Del-Campo et al.

2004), thus this respiratory cost results in an important sink for grapevines during the spring.

The study of the leaf growth pattern showed a slower LA expansion coupled to a faster DW and thickness increase in Grenache than in Tempranillo, which resulted in LMA values around 10% higher in mature leaves for Grenache than for Tempranillo. Previous results in a wider range of species showed that high values of LMA are commonly associated with high thickening of cuticle and epidermis (Niinemets and Sack 2006), consistent with a recent study by Tomás et al. (2014) in potted vines. Effectively, Tomás et al. (2014) showed that mature leaves of Grenache have thicker leaves (mesophyll and epidermis) than Tempranillo, highly due to a greater epidermal cell thickness (upper and lower epidermis). According to Kutschera (1992), outer cell layers constrain the extension of internal tissues and thus limit organ elongation. These greater epidermal cell thickness observed in Grenache could be linked to a higher limitation of the internal leaf tissues growth, probably due to a lower deformation of epidermal cell walls (leaf tissue less plastic), limiting the leaf expansion. Moreover, several authors reported a link between the leaf expansion rate and the cell-wall deformation properties, via the activities of enzymes under the control of chemical signals such as ABA (Dodd and Davies 1996, Salah and Tardieu 1997, Tardieu et al. 2010). In fact, (Martorell et al. 2015b) reported higher values of ABA in Grenache than in Tempranillo in an experiment with grapevines under field conditions. These facts all together could contribute to explain the differences in leaf expansion between cultivars in this study.

R_n is one of the important processes to be taken into account when calculating carbon balances in crops (Valentini, 2000), and this study is among few in the literature providing direct R_n measurements in field growing grapevines. In this experiment, the recorded R_n values agreed with previous reports for grapevines in different experimental locations (Schultz 1991, Gómez-Del-Campo et al. 2004, Escalona et al. 2012b, Zufferey 2016) showing high R_n rates associated to leaf expansion rates, followed by a deep decline when the leaf expansion finished. Focusing on this general response, the present results showed an important genotype effect on R_n rates, showing consistently higher respiratory losses in Tempranillo than in Grenache. According to the reported differences between cultivars, as well the known characteristic of Grenache as more drought tolerant than Tempranillo, a deeper study on the leaf characteristics and leaf

respiration can provide an interesting insight on the effects of leaf morphological characteristics on the respiratory cost of leaves. The results support that the growth costs of expanding leaves were associated to leaf morphology and growth rates (Figure 4). In this sense, the construction costs for a gram of leaf tissue was higher in Tempranillo than in Grenache, and this corresponds with a faster leaf expansion (high LA and low DW), and a higher leaf N concentration in Tempranillo than Grenache expanding leaves. Tomás et al. (2014) showed a higher mesophyll porosity and number of cell layers (spongy and palisade cells) in Tempranillo than other six grapevine cultivars. Therefore, the particular growth cost of leaf area unit could be linked to the type of mesophyll structure, as recently reported by (Ronzhina and Ivanov 2014). In addition, the higher N content observed on the Tempranillo expanding leaves could contribute to a higher metabolic machinery and consequently, to a higher growth costs of young leaves construction. However, the absence of a significant difference in maintenance costs of leaves between the two compared cultivars agrees with the absence of differences in N content in mature leaves. The relationship between foliar R_n and N concentrations has also been widely reported across different species and climates (Reich, Walters, Ellsworth, et al. 1998a, Atkin et al. 2015), and within varieties of a certain species (Bouma et al. 1992, Galmés et al. 2011, Laureano et al. 2013). It is well established that the relationships between tissue N and leaf CO_2 exchange rates are fundamental in plants due to the biochemical role of proteins in photosynthesis and respiration (Reich, Walters, Ellsworth, et al. 1998a). In this study, N content in young and mature leaves showed a significant correlation with R_n. Particularly in expanding leaves, the leaf N content expressed by dry weight explained most of the variability on R_n. These differences between cultivars in R_n along the leaf expansion will clearly account for the cost of canopy growth and maintenance in those varieties.

In conclusion, the correlation among leaf growth, leaf morphology and CO_2 exchange reported among different species (Reich, Walters, Tjoelker, et al. 1998), seem to be present among different cultivars in grapevine: some cultivars (as Tempranillo) produce expanding leaves with lower LMA, higher N content per dry weight unit, and greater respiration rates than some others (as Grenache), that have a higher LMA, less N content and lower respiration rates. In general, data from this study are consistent with the hypothesis that fast-growing cultivars have high tissue metabolic rates relative to characteristic of slow-growing ones. Tempranillo displayed high leaf growth rates (LA)

and associated traits, such as high respiration rates and N content, as well as leaf morphology (i.e. low LMA) associated with enhanced resource acquisition (i.e. high leaf area). These results reinforce the close coupling of tissue structural, chemical and metabolic characteristics within leaves that, in combination, are strongly associated with differences in growth rates. The type of leaf expansion (rapid or slow, Figure 2) seemed to play an important role in the leaf structure and as a consequence, in the respiratory rates and resources use efficiency (C and N) throughout the leaf expansion.

Acknowledgements

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CAPÍTULO 2

CHAPTER 2

Effect of genotype and plant water status on the whole cluster respiration throughout ripening in grapevines (Vitis vinifera L.)

Effect of genotype and plant water status on the whole cluster respiration throughout ripening in grapevines (*Vitis vinifera* L.)

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Introduction

The importance of crops in the sequestration of carbon is well recognize. In Europe, agriculture occupies almost half of the total land area and contributes significantly to global CO_2 emissions. Thus, due to growing concern about climate change, information on the contribution of several crops such as grapevines to carbon cycle is needed to accurately predict the CO_2 level in the atmosphere. This can be achieved by quantifying CO_2 fluxes from vineyards, taking into account the different components of the plant carbon balance under field conditions (Franck et al. 2011b, Escalona et al. 2016, Zufferey 2016, Hernández-Montes et al. 2017). The quantification of the CO_2 flows from each organ along the vine phenology contributes to calculate more accurate carbon balances, and also allows to understand plant physiological consequences of practicing different management techniques in vineyards (e.g. irrigation, cluster thinning, etc). Since most existing information on CO_2 exchange referred to leaves, data on the carbon budgets of stems, roots or fruits are rather scarce. Regarding the CO_2 fluxes from grapes, there are several studies that carried out experiments to study the contribution of grapevines berries to the carbon balance using potted plants grown under controlled conditions (Ollat and Gaudillère 2000, Palliotti and Cartechini 2005). Also, Palliotti and Cartechini (2001) studied that contribution under field conditions during the green stages of the berry development. To study the

contribution of the fruit to the carbon balance, is necessary to measure the fruit respiration and the fruit photosynthesis along the berry development. To date, some studies reported differences between the light and dark measurements in the green stages of the berry (Ollat and Gaudillère 2000, Palliotti and Cartechini 2001), which results in a photosynthetic capacity of the fruit. In this sense, fruit photosynthesis is drawing attention in the last years due to the photosynthetic properties of different tissues (exocarp, mesocarp and seeds) of green berries (Breia et al. 2013). According to Ollat and Gaudillère (2000), fruit photosynthesis supplied 10% of the carbon required for fruit development, and the whole berry development respiration accounted for 18% of the imported carbon in a potted experiment. Therefore, the study of respiration and photosynthesis of intact whole clusters under field conditions can led us to know the real contribution of fruit to the vine carbon balance in a commercial vineyard.

To date, studies of genetic variability have been aimed at improving the photosynthetic capacity of the leaves (Bota et al. 2001, 2016, Gomez-del-Campo et al. 2002). However, there are not many studies that explore the intra-cultivar variability in cluster respiration and photosynthesis, as well as the possibilities of improvement in this field. Only (Harris 1971) studied several grapevine cultivars, and they did not find any difference in the respiratory characteristics of berries between seeded and seedless cultivars and between pigmented and nonpigmented grapes. Thus, the study of CO_2 fluxes from grapes in different cultivars is interesting due to the worldwide genetic diversity in grapevines and the multitude of management techniques used in viticulture, in order to study the contribution of vineyards to the global carbon balance. Moreover, in grapevines, the effect of plant water availability on the CO_2 exchange has been studied among different cultivars in leaves (Escalona et al. 2003, Flexas et al. 2010), soil and roots (Hernández-Montes et al. 2017). However, the effect of vine water status on fruit CO_2 fluxes is still scarce, therefore, more studies in field grown grapevines are necessary. Moreover, the agronomic practices in viticulture can contribute to improve the carbon balance of berries (e.g. pruning, leaf removal, irrigation) by increasing the photosynthetic rates of fruit tissue in the first stages of the berry development (Dokoozlian and Kliewer 1996).

In this study, we examined the role of fruit CO_2 fluxes in the vine carbon balance using two different chambers (whole cluster respiration chamber and isolated berries respiration chamber), and two different cultivars (Grenache and Tempranillo)

under irrigation and non-irrigation conditions. Therefore, the objectives of this work were: (i) to determine the importance of agronomical management practices and plant architecture on fruit respiration under field conditions; (ii) to investigate the effect of cultivar and water status on fruit respiration and photosynthesis, and (iv) to determine the daily integrative carbon losses of clusters.

Materials and methods

Site description, plant material and treatments

This study was conducted in the experimental vineyard of the University of Balearic Islands (Palma, 39°38′17″N 2°38′54″E) during three consecutive years (2013, 2014 and 2015) using two *Vitis vinifera* grapevine cultivars widely cropped in Spain: Grenache and Tempranillo. Vines were planted in 2009 in rows (distance between rows was 2.5 m and between plants 1 m) and grafted onto rootstock 110 Richter. Vines were trained to bilateral cordons and spur pruned with an average of 12 buds per vine. Soil type was a typical clay-loam, general characteristics. Two treatments were imposed: irrigation (I), and non-irrigation (NI) consisted of withholding the drip irrigation during the whole growing season.

Weekly, irrigation doses were calculated from the ET_o registered by a meteorological station (Meteodata 3000, Geónica SA, Madrid, Spain) at the experimental site. The crop coefficient for the irrigation treatment (I) was initially fixed at 30% of ET_o in 2013, but in 2014 and 2015, it was increased to 40% to distinguish better the treatments. Vines were drip-irrigated (two or three irrigations per week) using three drip emitters per plant (4 L/h and spaced 0.3 m between drippers). Water status of I and NI vines was monitored at different phenological stages by measuring midday stem water potential (Ψ_{stem}) and predawn leaf water potential (Ψ_{pd}).

Measurement of midday stem water potential and predawn leaf water potential

Every 2 weeks (from May to September) Ψ_{stem} and Ψ_{pd} was measured with a Scholander pressure chamber (Soilmoisture Equipment, Goleta, CA, USA). Ψ_{stem} was measured on mature leaves that had been bagged with both plastic bag and aluminium foil 1 h before measurements. Ψ_{pd} was measured 1 h before sunrise on mature leaves. The measurements were carried out in four different plants per treatment on each sampling date.

Fruit respiration measurement

During 2013 and 2014, CO_2 efflux from entire clusters was measured using a homemade methacrylate respiration chamber (Figure 1A, 1B) directly connected to a portable gas exchange analyzer (Li-6400, LI-COR Biosciences, Lincoln, NE, USA). The entire cluster was measured on three dates along the berry development: hard-green (Keller et al. 2015), veraison and ripening stage. The measurement consisted in enclosing the cluster in the fruit chamber with the peduncle base outside using a small aperture in the chamber. Several discs were used to reduce the volume of the whole cluster chamber (Figure 1A, 1B). The possible gaps between the chamber and the base of the peduncle were covered with a contact rubber, to achieve total isolation of the chamber from the atmospheric air. All measurements were taken in the morning between 10:30 and 12:30 in four plants per treatment and cultivar. While measuring fruit respiration, fruit temperature inside the chamber was measured with a temperature probe connected to the LI-6400. The measurement protocol was fully automated. Three parameters were entered from the LI-6400 keypad to control the automatic measurement: ambient CO_2 concentration, the CO_2 change that determines the upper and lower set points and the number of measurement cycles per cluster. During measurements, the CO_2 concentration of the chamber air rises from the low set point, passing through the target ambient CO_2 concentration, to the high set point. Every two to three seconds, a flux is computed based on a running average of the rate of change of CO_2 concentration with time. This cycle was repeated three times, under light (L) (PAR around 1000-1200 μmol m^{-2} s^{-1}) and dark (D) conditions (by covering the fruit chamber with a reflective and thermally insulated sheet). The time needed to complete the cycle ranged from 4 to 8 min (L and D).

Figure 1 (A) Measurement of whole attached cluster respiration using a fruit respiration chamber connected to a portable gas exchange analyzer (LI-6400). **(B)** A close up of the fruit chamber with an attached cluster inside. **(C)** Measurement of the cluster volume using a non-destructive technique. **(D)** Measurement of detached isolated berries using a specific chamber (LI-6400-89 insect respiration kit) connected to a portable gas exchange analyzer (LI-6400).

All the CO_2 fluxes were referred to dry weight (DW) unit and fresh weight (FW) unit. To obtain the FW and DW of attached clusters, a linear regression between the cluster displaced volume and the cluster weight (fresh and dry) was made to refer the CO_2 efflux to fresh and dry weight unit (Figure 2). A regulated beaker and a known amount of water were used to measure the displaced volume of each cluster measured in the control plants (Figure 1C). To obtain the regressions, during each sampling date, the displaced volume was measured in several clusters from plants of each treatment and cultivar. Each cluster was weighted, and then dried for 72 hours in an oven at 70 ° C to obtain its dry weight. Regressions were performed (Figure 2A, 2B) between the volume, the dry weight and the fresh weight to obtain the estimated weight of the clusters measured with the fruit respiration chamber.

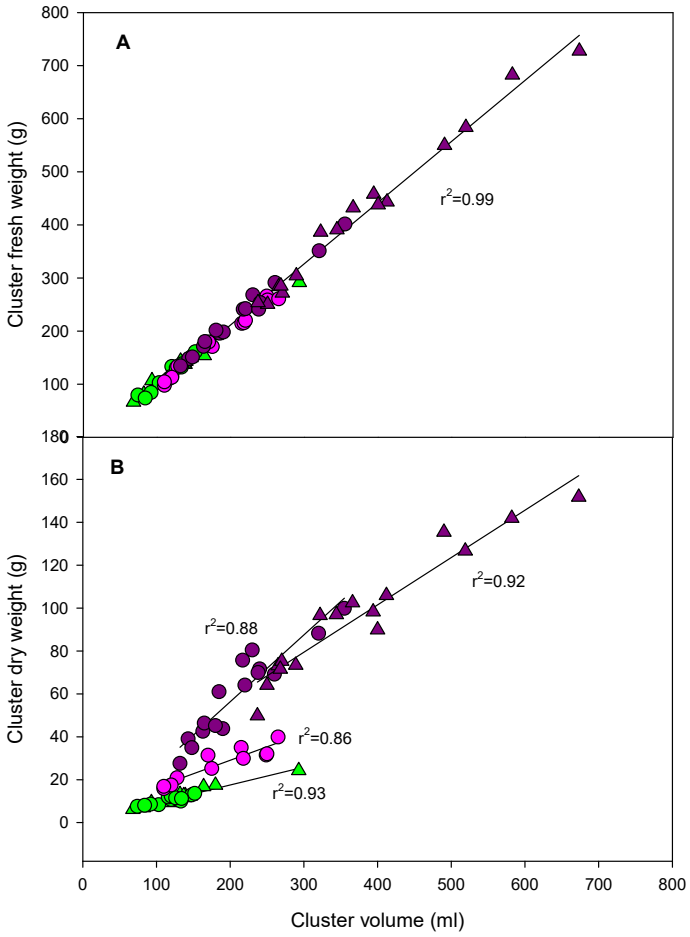

Figure 2. Relationship between cluster volume, cluster fresh weight and cluster dry weight during hard-green (green symbols), veraison (pink symbols) and ripening (purple symbols) stages of sampled Grenache (circles) and Tempranillo (triangles) clusters during the season 2013.

During 2015, fruit respiration was measured in the same stages than 2013 and 2014 (hard-green, veraison and ripening), but from samples of isolated berries (6-8 berries per sample) using a small chamber (6400-89 Insect Respiration Kit) connected to the LI-6400. The measurements were carried out under light and dark conditions, and the measurements were referred to FW and DW units. Thus, after each measurement, the diameter of the berries was measured, and the berries were weighted and dried in an oven (70 ºC for 72 h) in order to obtain the FW and DW of each sample.

Statistical analysis

Data were processed using ANOVA procedures, and means were separated by Tukey´s test. Multiple regressions and correlation analyses were set using JMP 12.2.0 (SAS Institute, Cary, NC, USA).

Results

Climatic conditions

Climatic conditions were recorded by a meteorological station located in the same field (Meteodata 3000, Geonica). The environmental conditions recorded were of typical Mediterranean Climate (Table 1), which included 2354 and 2474 and 2505 growing degree days (GDD) during the growing season in 2013, 2014, and 2015, respectively. Total rainfall from April to October (growing season) was 149 mm, 163 mm and 154 mm during 2013, 2014 and 2015 seasons respectively, with different pluviometry pattern among the years. The 2015 growing season was the driest of the three seasons because from the total rainfall (154 mm), it rained only 16 mm from April to July. During the summer, the mean monthly relative humidity was around 55- 70% in the three years. The average of maximum daily temperature reached was 32.4 °C in July 2013, 30.1 °C in August 2014 and 33.5 ºC in July 2015. Accumulated evapotranspiration was similar for the three years having only a certain decrease due to more frequent cloudy days in August 2013 and September 2014-2015. On an average, the irrigation applied during 2013, 2014 and 2015 was 95 mm, 177 mm and 221 mm, respectively.

Table 1. Environmental variables registered during 2013, 2014 and 2015. Subtotal represents the sum of the values from April to October (vine vegetative period). Abbreviations: Avg. T = Average of mean temperatures; Max. Temperature = Average of maximum temperatures; Min. Temperature = Average of minimum temperatures; GDD = Growing degree days calculated with a base temperature of 10 ºC; RH = Relative humidity; ETo = Reference evapotranspiration rate; P = Precipitation; I = Irrigation.

	Month	Avg. T (°C)	Max. T (°C)	Min. T (°C)	GDD	RH (%)	ETo (mm)	P (mm)	I (mm)
2013	April	14.4	19.8	8.8	132.4	69.2	95.6	52.1	
	May	16.6	21.1	11.1	203.1	65.3	118.5	4.9	
	June	21.3	27.1	14.7	340.5	57.8	146.5	2.4	3.9
	July	26.1	32.4	19.3	499.3	55.8	168.2	0.6	26.7
	Augost	25.7	31.2	19.7	485.2	59.4	128.4	45.0	31.1
	September	22.2	27.7	17.3	367.0	70.9	97.1	24.1	13.4
	October	20.5	25.7	15.8	326.0	74.0	69.5	19.9	21.5
	Subtotal				2353.5		823.8	149.0	96.5
2014	April	16.2	21.5	10.2	185.1	69.1	104.4	60.0	
	May	17.9	22.6	12.3	243.0	64.9	116.8	34.0	
	June	23.0	28.6	16.9	390.7	58.0	136.4	20.4	20.1
	July	24.7	29.5	19.1	455.0	58.4	157.2	0.9	82.4
	Augost	25.1	30.1	20.2	469.5	68.9	134.2	8.7	54.9
	September	24.0	29.1	19.5	421.1	74.5	94.7	30.8	13.5
	October	19.9	26.0	14.9	308.4	76.9	70.3	8.3	
	Subtotal				2472.8		814.0	163.1	170.9
2015	April	14.9	20.4	9.0	147.7	67.6	97.3	2.0	
	May	19.5	25.3	13.1	293.5	58.5	135.9	5.1	
	June	23.7	29.6	16.8	410.2	53.1	163.8	8.7	38.0
	July	27.9	33.5	21.8	555.0	54.6	174.8	0.0	109.8
	Augost	25.9	31.1	20.9	493.2	64.0	138.3	20.2	73.2
	September	21.5	26.0	17.1	344.5	77.4	89.6	78.9	
	October	18.4	22.7	14.3	261.4	78.2	59.2	38.8	
	Subtotal				2505.5		858.9	153.7	221.1

Leaf water potential

Midday stem water potential (Ψ_{stem}) was used as an indicator of plant water status during the experiments. Ψ_{stem} was recorded in Grenache and Tempranillo irrigated and non-irrigated plants during the main phenological stages (flowering, pea-size, veraison and ripening) of 2013, 2014 and 2015 seasons (Table 2). The ANOVA analysis showed no significant differences between cultivars until ripening stage, where Grenache showed a larger Ψ_{stem} than Tempranillo in all years. However, the analysis showed significant differences between treatments for Grenache and Tempranillo during the three years since the irrigation was started. The season 2014 registered the lowest Ψ_{stem} values for both Grenache and Tempranillo cultivars, due to the higher rainfall and the amount of irrigation water applied in this year (Table 1).

Table 2. Stem water potential measured in Grenache (G), Tempranillo (T), irrigated (I) and non-irrigated (NI) plants during the phenological stages of flowering, pea-size, veraison and ripening in the seasons 2013, 2014 and 2015. Different letters denote a statistically significant difference ($P<0.05$) between irrigation treatments.

			Ψ_{stem}			
Cultivar	Year	Treatment	Flowering	Pea-size	Veraison	Ripening
G	2013	I	0.77 ± 0.05 a	0.62 ± 0.02 b	1.01 ± 0.01 b	1.10 ± 0.09 a
		NI	0.89 ± 0.01 a	0.79 ± 0.02 a	1.26 ± 0.02 a	1.29 ± 0.03 a
	2014	I	0.47 ± 0.01 a	0.56 ± 0.07 b	0.62 ± 0.09 a	0.82 ± 0.09 b
		NI	0.48 ± 0.01 a	0.78 ± 0.04 a	1.32 ± 0.07 a	1.49 ± 0.04 a
	2015	I	0.66 ± 0.02 a	0.58 ± 0.02 b	0.86 ± 0.02 b	0.92 ± 0.05 b
		NI	0.70 ± 0.05 a	0.91 ± 0.03 a	1.12 ± 0.03 a	1.10 ± 0.03 a
T	2013	I	0.84 ± 0.05 a	0.70 ± 0.04 a	1.12 ± 0.04 b	0.82 ± 0.04 b
		NI	0.80 ± 0.01 a	0.65 ± 0.04 a	1.30 ± 0.01 a	1.04 ± 0.05 a
	2014	I	0.46 ± 0.05 b	0.43 ± 0.04 b	0.47 ± 0.06 b	0.53 ± 0.04 b
		NI	0.54 ± 0.01 a	0.88 ± 0.05 a	1.18 ± 0.06 a	1.16 ± 0.06 a
	2015	I	0.72 ± 0.04 a	0.67 ± 0.06 b	0.81 ± 0.08 b	0.82 ± 0.02 b
		NI	0.70 ± 0.02 a	0.91 ± 0.02 a	1.12 ± 0.02 a	1.06 ± 0.02 a

Berry development

The evolution of fruit size and weight was shown in Figure 3 for Grenache and Tempranillo, and for irrigated and non-irrigated vines. In general, the increase of berry diameter and fresh and dry weight was faster until veraison stage. After veraison, the weight and diameter of fruits slightly increased until the complete maturation stage. Tempranillo irrigated plants registered the highest berry size and weight. Significant differences were found between treatments since the pea size stage, where the irrigation was stablished.

Berry diameter (Figure 3A), berry fresh weight (Figure 3B), and berry dry weight (Figure 3C) of Grenache and Tempranillo increased at each development stage until the fruit completed the maturation process. The differences between cultivars in berry growth pattern were observed in veraison and ripening, where Tempranillo reached higher diameter, fresh weight and dry weight than Grenache. A clear effect of the irrigation treatments in the growth of the berries was observed from the first development stages until the maturation was completed.

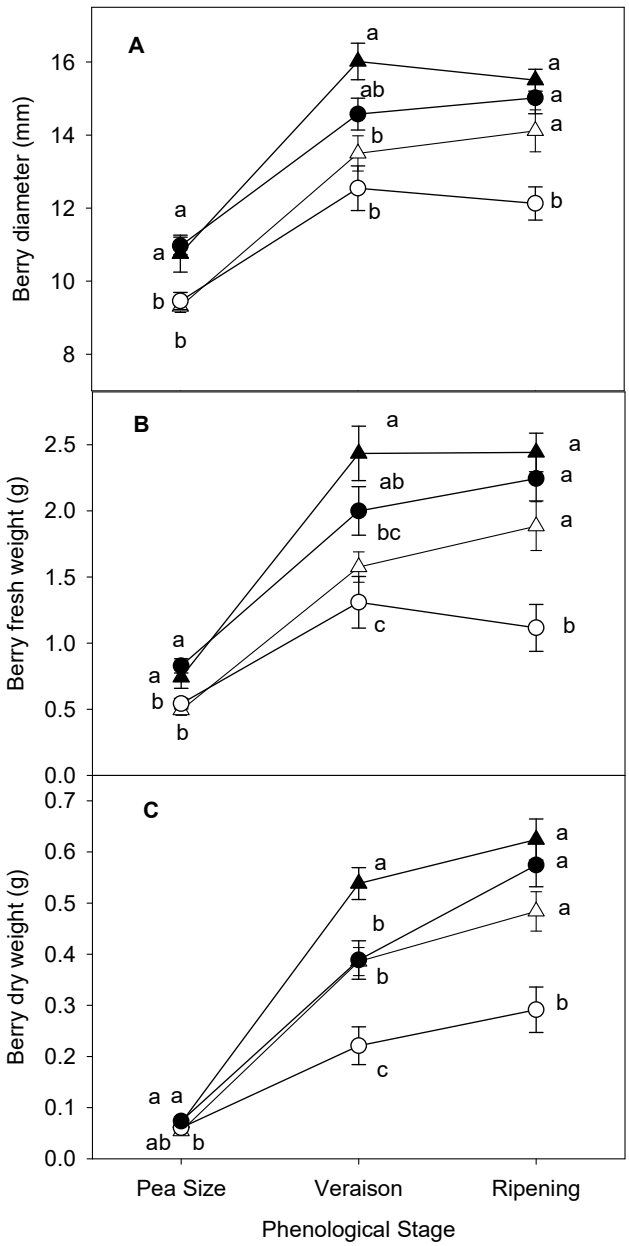

Figure 3. Evolution of diameter (A), fresh weight (B) and dry weight (C) of berries from Garnacha (circles) and Tempranillo (triangles) irrigated (closed symbols) and non-irrigated (open symbols) plants. Values are means±SE of 4 replicates. Different letters denote a statistically significant difference by a Tuckey´s test ($P<0.05$) among cultivars and irrigation treatments.

Fruit CO_2 fluxes along grape development

Fruit CO_2 fluxes were studied under light and dark conditions at three different berry development stages (hard-green, veraison and ripening) to determine the CO_2 losses from clusters measured with two different techniques under field conditions. Figure 4 shows the averages of fruit CO_2 fluxes expressed per dry mass unit at each

development stage, in Grenache and Tempranillo irrigated and non-irrigated vines. On the one hand, during 2013 (Figure 4A, 4D, 4G) and 2014 (Figure 4B, 4E, 4H), fruit respiration was measured using a whole cluster chamber that enclosed intact clusters. On the other hand, during 2015 (Figure 4C, 4F, 4I), isolated sampled berries were measured in a small chamber. In general, the fruit respiration per dry mass unit decreased from the hard-green stage of the berry until its maturation was completed, in the three years and using both techniques (whole cluster chamber and isolated berries chamber).

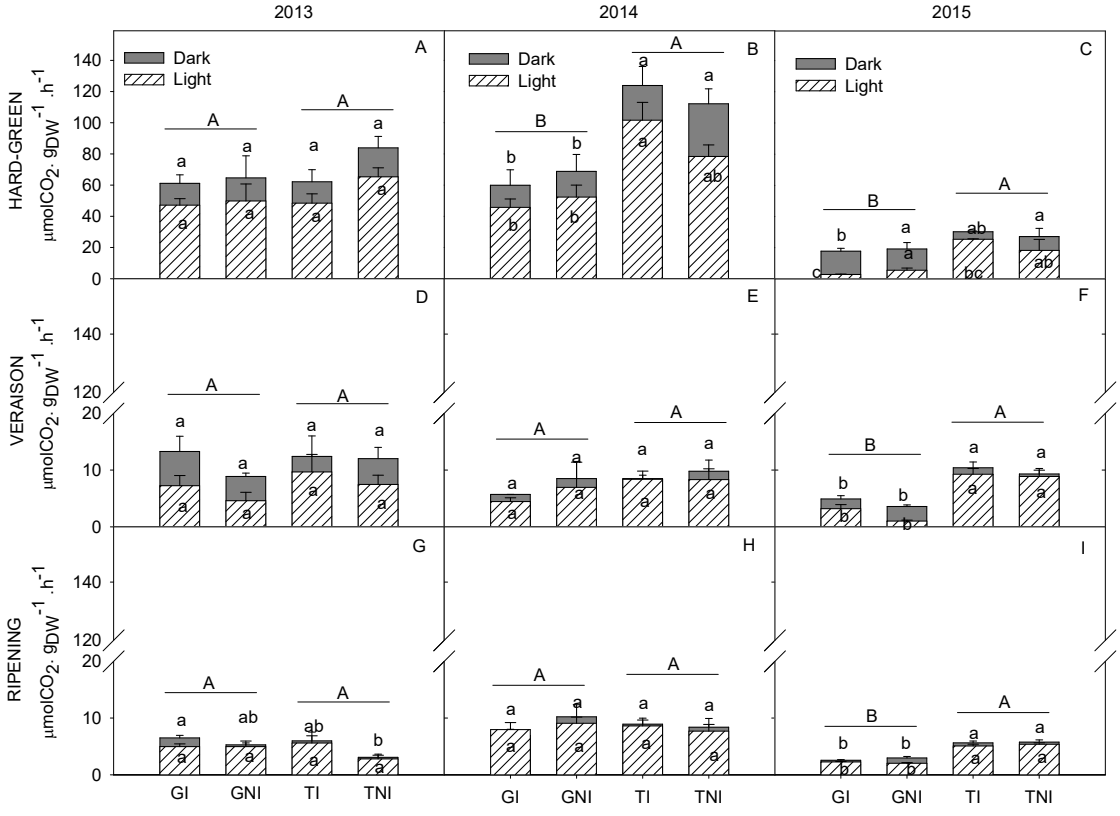

Figure 4. Average CO_2 efflux per dry weight unit from fruits measured in irrigated (I) and non-irrigated (NI) Grenache (G) and Tempranillo (T) plants during three development stages of the berry (hard-green, veraison and ripening) in the seasons 2013 (A, D, G), 2014 (attached whole clusters) and 2015 (detached isolated berries). Values are means±SE of 4 replicates. Different letters denote a statistically significant difference by a Tuckey´s test ($P<0.05$) among cultivars and irrigation treatments (small letters) and between cultivars (capital letters).

A global statistical analysis of the data showed that the total average of fruit CO_2 fluxes from the year 2015 (isolated berries chamber) was significantly lower than the other two years (2013 and 2014, whole cluster chamber) both in L (65% lower) and D (70% lower) conditions. Moreover, significant differences were found among berry

development stages (hard-green, veraison and ripening) in every year, being the hard-green stage the moment of highest CO_2 losses from the fruits. These differences led us to analyze the data from each berry development stage and each year, separately. The statistical analysis of hard-green stage (Figure 5A, 5B, 5C) showed significant differences in fruit CO_2 fluxes among years under both L and D conditions, registering 2014 the highest values, followed by 2013 and 2015. Also, at the same stage (hard-green) significant differences were found between cultivars in 2014 and 2015: during 2014, Tempranillo reached CO_2 fluxes 80% larger than Grenache in both L and D conditions, and during 2015, the increase of Tempranillo respect to Grenache was higher in L (95%) than D (55%). These differences between cultivars were maintained only in 2015, for both L and D conditions, along veraison (Figure 5F, Tempranillo 331% higher than Grenache in L, Tempranillo 143% higher than Grenache in D) and ripening (Figure 5I, Tempranillo 132% higher than Grenache in L, Tempranillo105% higher than Grenache in D).

The net photosynthesis rates from clusters and berries were calculated from the difference between the CO_2 flux in D and the CO_2 flux in L (Figure 5). The statistical analysis of the photosynthesis capacity of fruits showed the same behavior than respiration rates, previously explained above. Significant differences in fruit photosynthesis were observed in hard-green, veraison stage during 2013 and 2015 years.

In the context of irrigation treatments, the ANOVA analysis did not show any significant difference between irrigated plants and non-irrigated plants, at any berry development stage in any year.

The relationship between the cluster weight and the respiration rates followed an exponential function for 2013 and 2014 seasons. Also, a similar relationship was found for the berry weight and the fruit respiration for the season 2015.

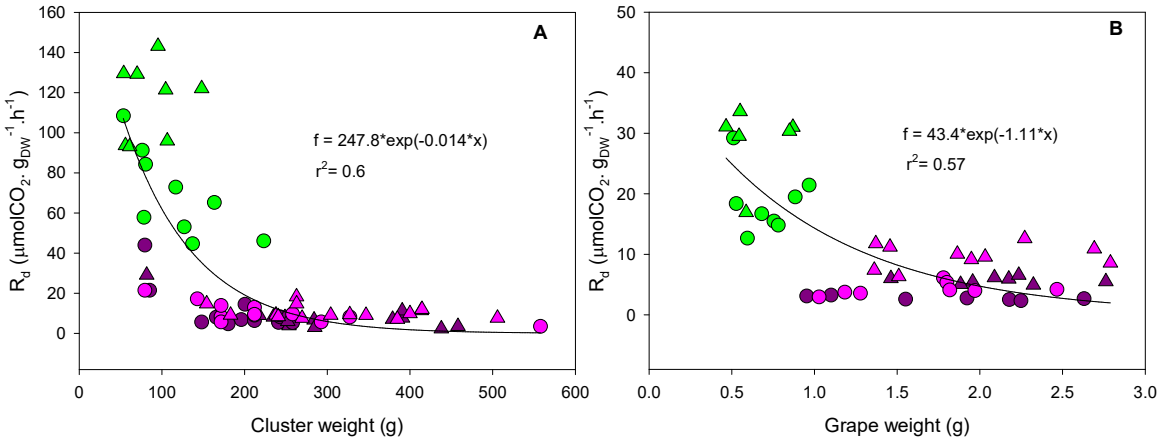

Figure 5. (A) The relationship between cluster weight and CO_2 efflux referred per dry weight during 2013 and 2014. **(B)** The relationship between berry weight and CO_2 efflux referred per dry weight during 2015.

Integrated carbon losses

The measurements under L and D conditions led us to calculate the carbon losses from fruits during a whole day (day and night). Figure 6 showed the integrated values of carbon losses per day for each berry development stage and for irrigated and non-irrigated Grenache and Tempranillo vines, an average in 2013-14 (Figure 6A) and 2015 (Figure 6B). The pattern was the same that the fruit CO_2 fluxes, with higher values were registered during hard-green, after where, the fruit carbon losses were stabilized (around 0.5 g. C. Kg^{-1}. day^{-1}). The ANOVA analysis showed significant differences only between cultivars.

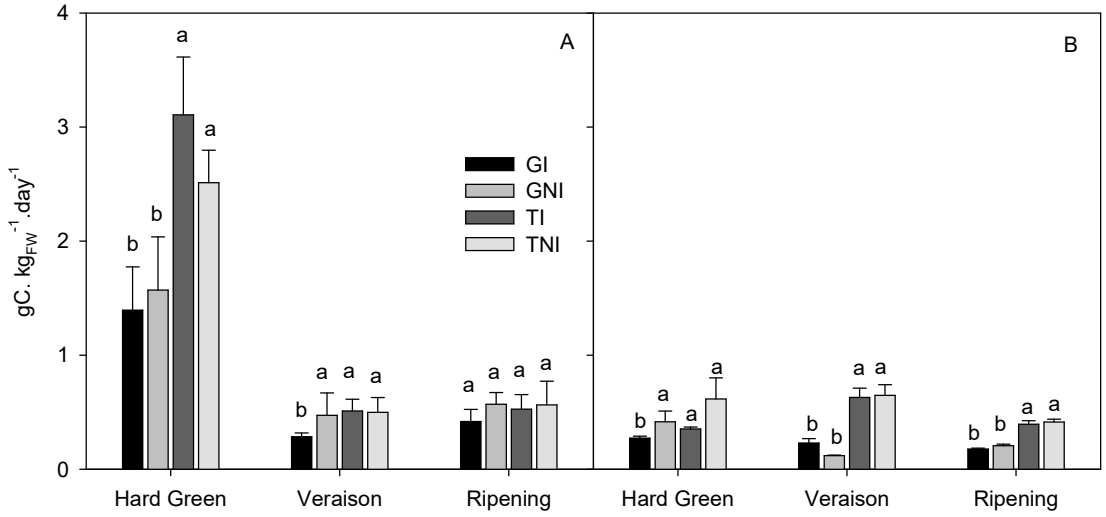

Figure 6. Carbon losses per day and kilogram of fruit at hard-green, veraison and ripening stage during 2013-2014 (A) and 2015 (B) for irrigated and non-irrigated Grenache and Tempranillo plants. Different letters denote a statistically significant difference by a Tuckey´s test ($P<0.05$) among cultivars and irrigation treatments.

Discussion

In this study, fruit respiration has been measured in whole clusters (2013 and 2014) and isolated berries (2015) under L and D conditions. In general, the results from this study are in agreement with previous reports, where the grape berry CO_2 fluxes at the first stages of development were high (45 umolC.g_{FW}^{-1}.h^{-1} at 10 days after anthesis), and decrease to 10 umolC.g_{FW}^{-1}.h^{-1} 10 days before veraison (Palliotti and Cartechini 2001) in a Cabernet Sauvignon experiment under field conditions. A similar decline was also observed by Ollat and Gaudillere (2000), who reported respiration rates of 45 umolCO_2.g_{DW}^{-1}.h^{-1} at 20 days after anthesis to decrease until 5 umolCO_2.g_{DW}^{-1}.h^{-1} at 100 days after anthesis in an experiment using Cabernet Sauvignon cuttings. Although the fruit respiratory pattern was similar, absolute values were different due to the use of different techniques to measure fruit CO_2 exchange (Figure 4). Whole cluster respiration was measured during 2013 and 2014, and isolated grape berries were measured during 2015. Statistical analysis between different years can give us information about the consequences of measuring with one technique or another. The differences found between years were accentuated when the berry was green and hard. In this grape development stage (hard-green), the values of CO_2 flux referred to dry weight registered during 2013 and 2014 (whole clusters) were significantly higher than those obtained in 2015 (isolated berries), under L and D conditions. In this sense, it should be pointed out that the whole cluster measurements involve measuring the rachis and the grapes at the same time, and this fact could cause those differences. There is no information about rachis respiration, but it is reported the chlorophyll content of young and old rachis tissue (Palliotti and Cartechini 2001), which showed concentrations of chlorophyll a and b equal to 7% respect to the chlorophyll concentration in main leaves. The same study showed the CO_2 exchange of young and old tendrils under light and darkness, similar to berries 10-20 days after anthesis. Also, Aschan and Pfanz (2003) reported that non-foliar tissues, as green stems, contribute an important proportion to whole-plant carbon gain. They found that some properties of the photosynthetic apparatus of leaves, like abundant stomata in the epidermis and similar response curves to environmental factors such as light, are identical in green stems (Aschan and Pfanz 2003). Although the respiratory and photosynthetic potential of the rachis is unknown, this study reveals the possible importance of this tissue as a carbon source for the grapes, due to its low dry weight (14% respect to 28% in leaves (Palliotti and Cartechini 2001)), the high nitrogen

content (20 mg/g respect to 28 mg/g in leaves (Palliotti and Cartechini 2001) and the large difference between the measurements in the darkness in whole clusters (rachis and berries) and isolated berries. Moreover, these differences between techniques were maintained under L conditions in the hard-green stage, and this fact has direct relationship with the light interception inside each chamber. Using the whole cluster chamber, the real conditions in the canopy are kept, and the radiation reaches only one side of the cluster. The remaining surface of the berries and the rachis are shaded or partially shaded by other berries or by leaves. Thus, cluster carbon balance under field conditions seems that depend on factors as row orientation, canopy management or berry size. However, using the chamber for the respiration of isolated berries respiration, the light interception of grapes is homogeneous, and the shading between them is almost null. Therefore, the measurements under L conditions are significantly lower in this case (isolated berries) than in the previous one (whole cluster) because more grape tissue is recycling CO_2 due to the sunlight. In this study, the photosynthesis was calculated as the difference between the CO_2 efflux under D and under L. Under L conditions, the recycled CO_2 accounted for around 20-30% of the total CO_2 respired in darkness. This percentage was higher only in Grenache (70-80%) when measuring isolated berries, in line with the results of Ollat and Gaudillère (2000), that found a recycling of 70% of the carbon used for respiration in attached clusters of Cabernet Sauvignon cuttings. These results suggest that fruit photosynthesis could be a candidate to increase the efficiency in the use of resources in vineyards by managing the light interception of clusters during the day, in order to get a direct supply of carbohydrates reducing the high transport costs from leaves. Palliotti and Cartechini (2001) reported the light curves of flowers and green berries, which confirms the effect of light on cluster photosynthesis.

In veraison, the CO_2 efflux decreased until 10 $umolCO_2.g_{DW}^{-1}.h^{-1}$ and the differences among years were lower in this stage. Also, the differences between the measurements under light and under darkness decreased, but it was variable due to the heterogeneity of berry characteristics during veraison. Ollat and Gaudillère (2000) reported that the differences between the respiration rates under D and L were gradually diminishing as the berries matured. However, Palliotti and Cartechini (2001) showed little difference between L and D measurements from early stages of berry development (before veraison). Finally, during ripening, the differences between the CO_2 fluxes

under L and under D conditions were very low or almost null, in line with the results of Ollat and Gaudillère (2000) from a Cabernet sauvignon potted experiment.

In this study, the fruit CO_2 fluxes showed significant differences between cultivars in 2014 (hard-green stage) and 2015 (hard-green, veraison and ripening stages). Measuring isolated berries during 2015, Tempranillo registered values 55%, 140% 110% higher than Grenache in hard-green stage, veraison and ripening, respectively. This sustained increase of fruit respiration in Tempranillo regarding to Grenache resulted in higher C losses when the values were integrated all along the day (Figure 6). However, the daily integrated losses of carbon during 2013 and 2014 showed higher values than during 2015. This is related to the importance of choosing the correct technique when estimating the real carbon losses under field conditions. These results suggest that the integrated carbon losses can be underestimated measuring isolated berries before veraison stage.

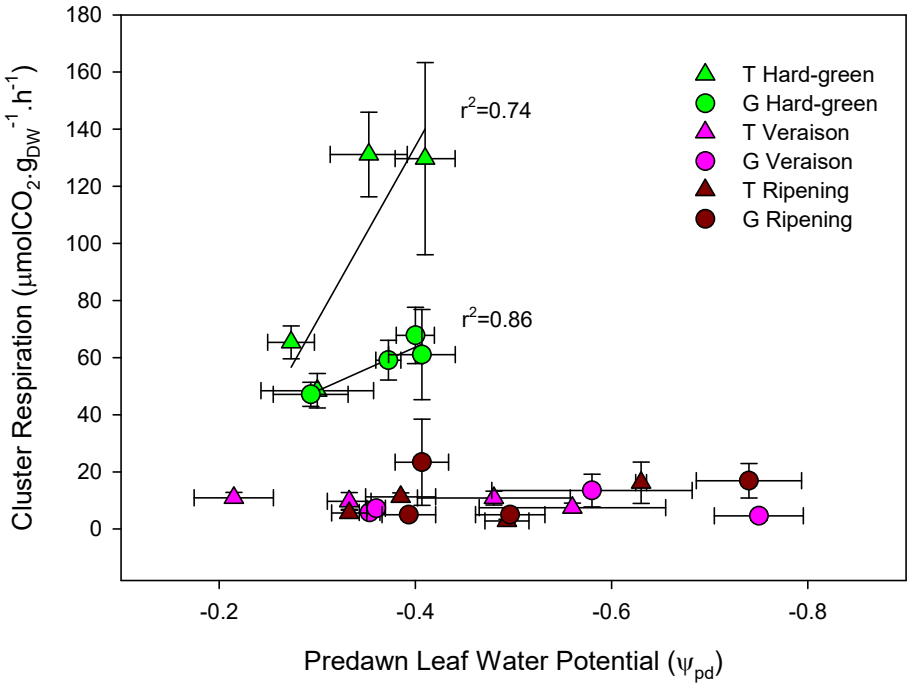

Figure 7. Relationship between plant water status and cluster CO2 efflux measured during 2013 and 2014 at hard-green, veraison and ripening stage.

In terms of irrigation effect on fruit respiration along the grape berry development, there were no significant differences between treatments in any year of measurement. However, a relationship was found between predawn leaf water potential and respiration, only in the hard-green stage (Figure 7). This suggests that the low level

of water stress at this time (before veraison) made the differences less visible, but it seems that it may be decisive to take into account the water status of plants when determining a more accurate carbon balance when calculating respiratory losses by cluster respiration. The hard-green stage implies high relative growth rates that can be influenced more importantly by water stress. All this suggests that more studies are necessary to study deeply the effect of water stress on fruit respiration.

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CAPÍTULO 3

CHAPTER 3

Influence of water availability and grapevine phenological stage on the spatial variation in soil respiration

Influence of water availability and grapevine phenological stage on the spatial variation in soil respiration

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Running title: Water status and vine phenology affect soil respiration

Abstract

Background and Aims: An understanding of spatial variation in soil respiration is critical to determining the carbon balance in grapevines. The effect of soil water content on soil respiration during different phenological stages in two grapevine cultivars, Grenache and Tempranillo, was studied over two seasons (2013 and 2014).

Methods and Results: Soil respiration was measured from five locations confined to within and between rows of vines at five phenological stages between budburst and postharvest under irrigated and non-irrigated conditions. Vine phenology influenced the in-row soil CO_2 efflux to a greater extent than the between-row CO_2 efflux, while irrigation resulted in 65% higher in-row soil respiration than that of the between-row positions. In contrast, the flux from in-row position of the non-irrigated treatment was only 25% higher than the between-row position. Soil moisture and vine phenological stages appeared to have a greater influence on soil respiration than soil temperature.

Conclusions: Significant correlations existed among soil respiration, irrigation and the vine phenological stages. Soil respiration increased from budburst to pea-size berry stage, thereafter it decreased until ripening stage before increasing again during postharvest stage.

Significance of the Study: The study showed that soil water availability and vine phenology play an important role in influencing soil respiration under field conditions.

Keywords: carbon balance, climate change, grapevine, irrigation, soil CO_2 efflux

Introduction

Global warming simulations predict a general water scarcity and a progressive increase of CO_2 in the atmosphere in the Mediterranean region which have led in recent years to increased studies on carbon balances in different ecosystems (oceans, forest and grassland) ((Bousquet et al. 2000, Valentini 2000, Flanagan et al. 2002, Piao et al. 2009). Agriculture, especially vineyards, are an important component of the Mediterranean-climate ecosystems. In Europe, agriculture occupies large tracts of land, reaching almost half of the total land area (Stoate et al. 2009), and contributes significantly to global CO_2 emissions. Consequently, several organisations have developed strategies to promote carbon sequestration in different crops to mitigate the global climate change effects (Intergovernment Panel on Climate Change 2014). The agricultural land area in Spain accounts for almost half of the total land surface (Instituto Nacional de Estadistica 2015) wherein the vineyards cover over 950,000 hectares, rendering the country as the largest in terms of vineyard area both in Europe (30% of total surface) and in the world (13.4% of total surface) (Organisation Internationale de la Vigne te du Vin 2013). Historically, irrigation has been banned by the wine laws of the European Union, however, in recent years, countries such as Spain have been relaxing their regulations in view of increased frequency, extent, and intensity of high temperature (heat wave) coupled with variations in precipitation and seasonality. Heat waves during the growing season not only restrict vine productivity but also raise soil temperature causing greater loss of water from the soil (Schultz 2014). These unprecedented climatic changes necessitate irrigation to be an integral component of production practices so that grape production can be sustained under stressful circumstances.

In Europe (Spain), areas planted with grapevines have been largely ignored in terms of carbon flux monitoring and carbon budget assessment. In this era of unprecedented climate change, information on CO_2 fluxes from additional sources such as the vineyard is needed to accurately quantify the CO_2 level in the atmosphere. This can be achieved by quantifying CO_2 fluxes and carbon balances from the vineyard soils. Soil respiration is the second largest component determining carbon flux of terrestrial ecosystems after photosynthesis (Raich and Schlesinger 1992). For instance, soil

respiration accounts for up to 75% of total net carbon fixed by photosynthesis in grapevines (Escalona et al. 2012b). Soil respiration components are usually separated into: (i) respiration from roots, exudates, mycorrhizae and microorganisms in obligate associations [autotrophic respiration (Ra)]; and (ii) respiration from soil microorganisms that subsist on the decomposition of soil organic matter [heterotrophic respiration (Rh)] (Scott-Denton et al. 2006). The spatial and temporal fluctuations in both components have been widely studied in grasslands (Baggs 2006, Kuzyakov 2006), forests (Davidson 2006) and several crops (Buwalda 1993, Hao 2014 (Buwalda 1993). Also, several parameters were studied to find the factors that affect soil CO_2 emissions, such as soil physical properties, soil biochemical properties, environmental conditions or crop management techniques (Allaire et al., 2015). Conversely, few studies have focussed on soil and root respiration of grapevines (Franck et al. 2011, Lardo et al. 2015). Therefore, more information is needed for a deeper understanding of the temporal and spatial variability in soil CO_2 dynamics, and for accurately estimating vine carbon balances under field conditions. Accordingly, this research focused on examining Ra and Rh by measuring soil respiration at different locations during different phenological stages of grapevines under irrigated and non-irrigated conditions.

Water availability in soil significantly influences soil respiration (Davidson et al. 1998, Kelting et al. 1998, Wan et al. 2007, Perez-Quezada et al. 2012). According to Wan et al. (2007) soil water content is responsible for around 56% of the changes in soil respiration. In grapevines, Lardo et al. (2015) reported an increase of 300% in total soil respiration a week after supplemental irrigation in a field experiment. In contrast, Escalona et al. (2012) observed 140–170% increase in root respiration of water-stressed grapevines. From these studies, it is clear that our understanding of how irrigation affects vine root growth, activity and distribution has greatly increased (Van Zyl 1984, Bassoi et al. 2003, Soar and Loveys 2007, Comas et al. 2010). There is little information available, however, on the dynamics of root activity and soil respiration in relation to phenology under different soil water content in grapevines.

As a consequence, due to the importance of soil respiration in calculating carbon balances of vineyards, and because of the lack of information on the dynamics of soil respiration under field conditions, the objectives of this work were to: (i) examine the spatial variability in soil respiration in a vineyard; (ii) investigate the changes in soil

respiration in relation to vine phenology; and (iii) determine the influence of soil water content on soil respiration.

Materials and methods

Site description, plant material and treatments

This study was conducted in the experimental vineyard of the University of Balearic Islands (Palma, 39°38′17″N 2°38′54″E) during two consecutive years (2013 and 2014) using two *Vitis vinifera* grapevine cultivars widely cropped in Spain: Grenache and Tempranillo. Vines were planted in 2009 in rows (distance between rows was 2.5 m and between plants 1 m) and grafted onto rootstock 110 Richter. Vines were trained to bilateral cordons and spur pruned with an average of 12 buds per vine. Soil type was a typical clay-loam, maintained free of weeds by surface tillage to facilitate measurement of soil respiration. Soil physical and chemical characteristics are reported in Table 1. Main vine growth stages were identified using the modified E-L system (Coombe and Dry 2005) (Table 2). Two treatments were imposed: irrigation (I), and non-irrigation (NI) consisted of withholding the drip irrigation during the whole growing season.

Table 1. Physical and chemical properties of the field soil.

	Soil depth (cm)		
	0−29	**30−85**	**85−165**
Physical parameters			
Coarse elements (g/kg)	169.5	123	171.95
Apparent density	1.4	1.4	1.4
Sand (g/kg)	314.3	212.5	338.7
2−1 mm (g/kg)	58.6	36.75	57.45
1-0.5 mm (g/kg)	64.75	42.35	65.7
0.5−0.25 mm (g/kg)	52.1	38.2	54.25
0.25−0.1 mm (g/kg)	73	47.75	80.3
0.1−0.05 mm (g/kg)	65.8	47.5	81
Silt (g/kg)	408.85	426.45	335.75
0.05−0.02 mm (g/kg)	136.45	119.9	112.5
0.02−0.002 mm (g/kg)	272.4	306.5	223.25
Clay (g/kg)	276.95	361.05	325.6
Texture	Clay-loam	Clay-loam	Clay-loam
Chemical parameters			
Cation-exchange capacity (g/kg)	542.45	376.9	600.4
$CaCO_2$ (g/kg)	139.95	92.2	111.8
pH H_2O (1:2,5)	8.15	8.35	8.35
pH KCl 1mol/L (1:2.5)	7.65	7.55	7.65
EC 1:5 (dS/m)	0.095	0.06	0.065
Total organic C (g/kg)	17.5	12.7	8.8
FC -33 kPa (g/kg)	213.7	202.25	256.55
PWP -1500 kPa (g/kg)	98.35	119.75	118.3

EC, soil electrical conductivity; FC, field capacity; PWP, permanent wilting point.

Weekly irrigation doses were calculated from the ET_o registered by a meteorological station (Meteodata 3000, Geónica SA, Madrid, Spain) at the experimental site. The crop coefficient for the irrigation treatment (I) was initially fixed at 30% of ET_o in 2013, but in 2014, it was increased to 40% to distinguish better the treatments. Vines were drip-irrigated (two or three irrigations per week) using three drip emitters per plant (4 L/h and spaced 0.3 m between drippers). Water status of I and NI vines was monitored at different phenological stages by measuring soil water content and predawn leaf water potential (Ψ_{pd}).

Measurement of soil moisture and predawn leaf water potential

Soil water content measurements were performed using a capacitive probe (PR2 HH2 Soil Moisture Profile, Delta-T Devices, Cambridge, England). Starting at pre-budburst stage, soil moisture was measured every 2 weeks at 0.1, 0.2, 0.3, 0.4, 0.6 and 1m depth. Data were collected using a data logger (DL6 Data Logger, Delta-T Devices, Cambridge, England) connected to the probe. Measurements were made on three I and

three NI vines per cultivar, the same as soil respiration measurements. Two tubes were installed along the row, one close to the trunk and the other one was placed 0.5 m from the trunk. The field capacity (FC) and the soil permanent wilting point (PWP) were calculated from soil analyses to reset the available soil water to field capacity.

Every 2 weeks Ψ_{pd} was measured with a Scholander pressure chamber (Soilmoisture Equipment, Goleta, CA, USA). The measurements were made 1 h before sunrise on four mature leaves of different plants per treatment during the growing season.

Soil respiration measurement

Soil respiration was measured with a soil respiration chamber (Li-6400-09) directly connected to a portable gas exchange analyser (Li-6400, LI-COR Biosciences, Lincoln, NE, USA). While measuring soil respiration by placing this chamber on a polyvinyl chloride collar fixed to the soil, soil temperature at 0.15 m depth was measured with a soil temperature probe. All measurements were made in the morning between 1000 and 1200 at budburst, flowering, pea size, ripening and postharvest stages (Table 2). Soil respiration was measured from three positions along three vines (positions 1, 2 and 3; Figure 1) per treatment and at two positions from between rows (positions 4 and 5; Figure 1).

Statistical analysis

Data were processed using ANOVA procedures, and means were separated by Tukey´s test. Multiple regressions and correlation analyses were set using JMP 12.2.0 (SAS Institute, Cary, NC, USA).

Table 2. Phenological stages and corresponding dates for 2013 and 2014.

		Date	
Phenological stage	**Phenological stage (Eichhorn and Lorenz)†**	**2013**	**2014**
Budburst (two to three leaves separat	9	≈10 April	≈01April
Start flowering	20-21	≈15 May	≈15 May
Flowering	23	≈31 May	≈04 June
Pea size	31-32	≈08 July	≈05 July
Veraison	35	≈29 July	≈05 August
Ripening	38	≈22 August	≈27 August
Postharvest	41	≈01 October	≈28 Septembe

†Coombe and Dry (2005).

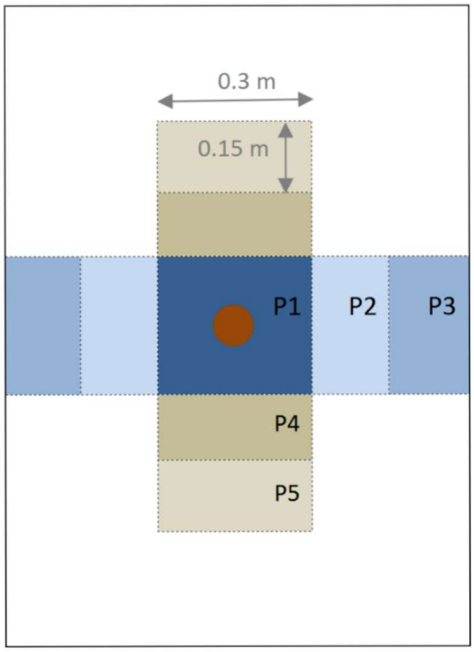

Figure 1. Diagrammatic representation of different locations to measure soil respiration. The brown circle represents vine trunk. P1, P2 and P3 represent the locations along the irrigation line, and P4 and P5 represent the locations between the rows.

Results

Environmental conditions

The environmental conditions recorded were of typical Mediterranean climate, which included 2354 and 2474 growing degree days (GDD) during the growing season in 2013 and 2014, respectively (Table S1). Total rainfall from April to October (growing season) during 2013 and 2014 seasons was 149 mm and 163 mm, respectively, and the evaporative demand (ET_o) was 823 mm and 814 mm, respectively (Figure 2). During the summer, the mean monthly RH was 55–60% in 2013, and 58–70% in 2014. The daily average temperature reached 32.4°C in July 2013 and 30.1°C in August 2014. Precipitation was low during these months (0.6 and 8.7 mm in 2013 and 2014, respectively). On an average, the irrigation applied during 2013 and 2014 was 95 mm and 177 mm, respectively, whereas the total amount of water applied (sum of precipitation and irrigation) was 35% of total ET_o.

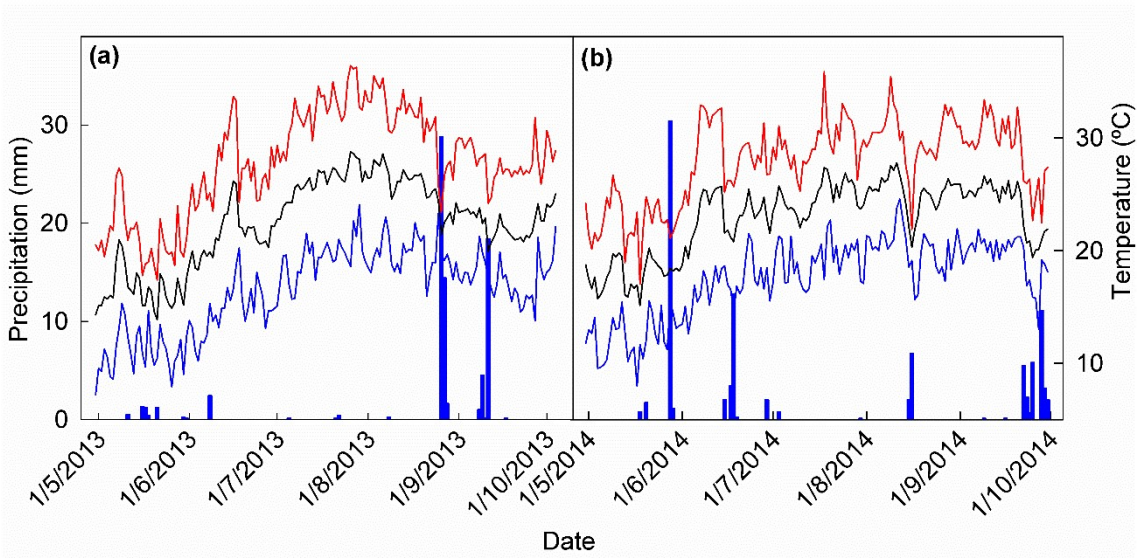

Figure 2. Meteorological variables of the vineyard during the growing season (2013-2014): mean (——), minimum (——) and maximum (——) daily air temperature (Ta). Vertical bars indicate precipitation.

Soil water content and Ψ_{pd}

Soil water content (SWC) was recorded in I and NI vines during 2013 and 2014 (Figure 3). Soil water content measured at 0.3–0.4 m depth in the I vines was almost constant during the irrigation period for Grenache and Tempranillo. In the NI vines SWC, however, showed a steady decline from May in both years when temperature was higher and rainfall was scarce. Differences between treatments were observed in both years. In 2014 season (Figure 3b,d), the difference between treatments was greater than in 2013 season (Figure 3a,c) due to an increased amount of irrigation water applied.

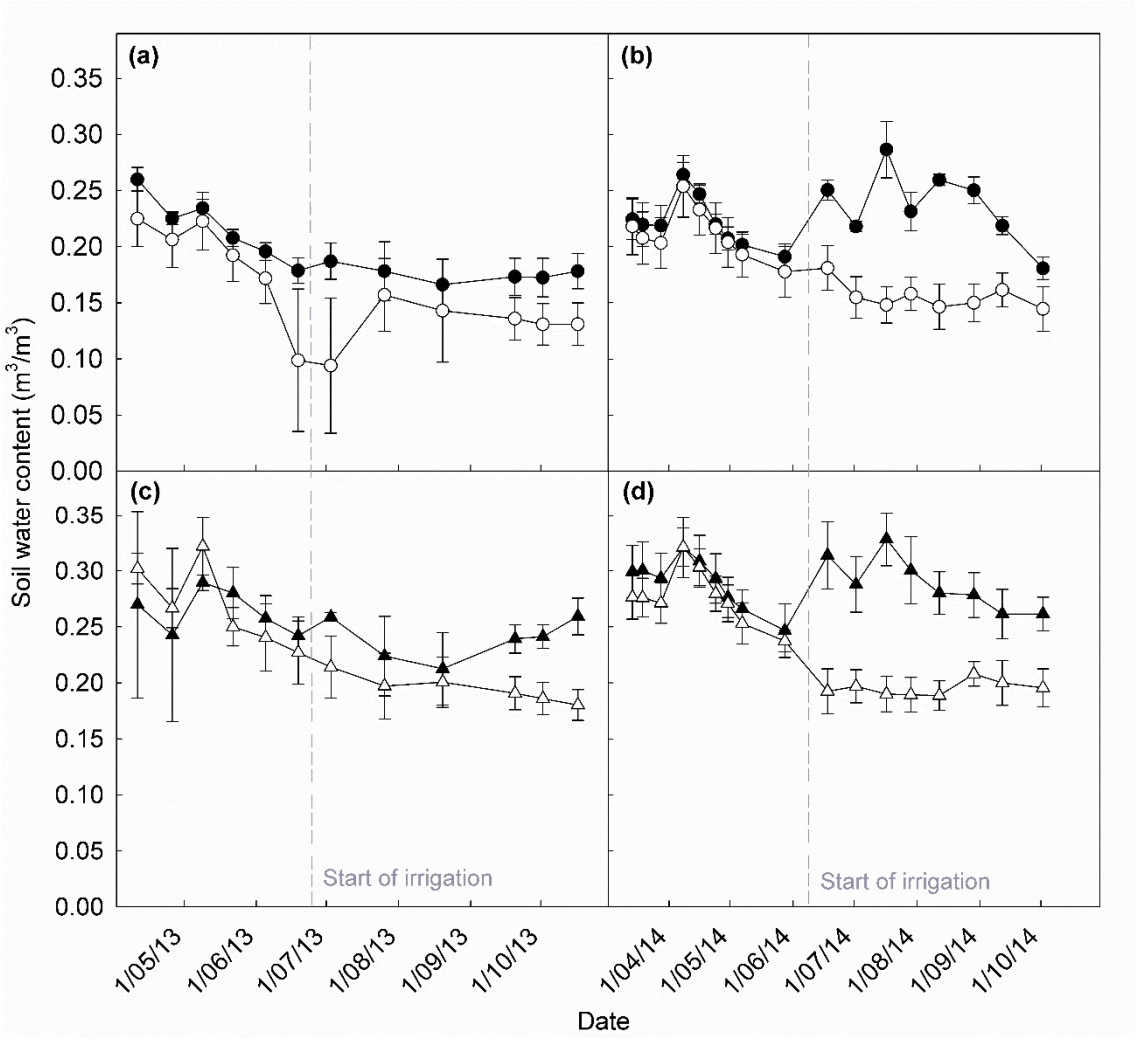

Figure 3. Soil water content measured at 30–40 cm depth in irrigated (●) and non-irrigated (○) Grenache vines, and in irrigated (▲) and non-irrigated (△) Tempranillo vines during (a, c) 2013 and (b, d) 2014 seasons. Bars indicate ± SE (*n*=3).

Measurement of Ψ_{pd} (Figure 4) showed differences between treatments from the beginning of veraison (July) to harvest (September) possibly due to differences in leaf area (data not shown) and water demand between treatments at this stage. The Ψ_{pd} of Irrigated vines was higherhan -0.4 MPa during the 2013 (Figure 4a) and 2014 (Figure 4b) growing seasons. Moreover, NI vines showed a progressive decline in plant water status from veraison (around -0.5 MPa) to ripening (around -0.7 MPa) during 2013, and from veraison (around -0.4 MPa) to harvest (around -0.7 MPa) during 2014 in both cultivars, reflecting the constant soil water depletion until the end of summer (Figure 4a,b).

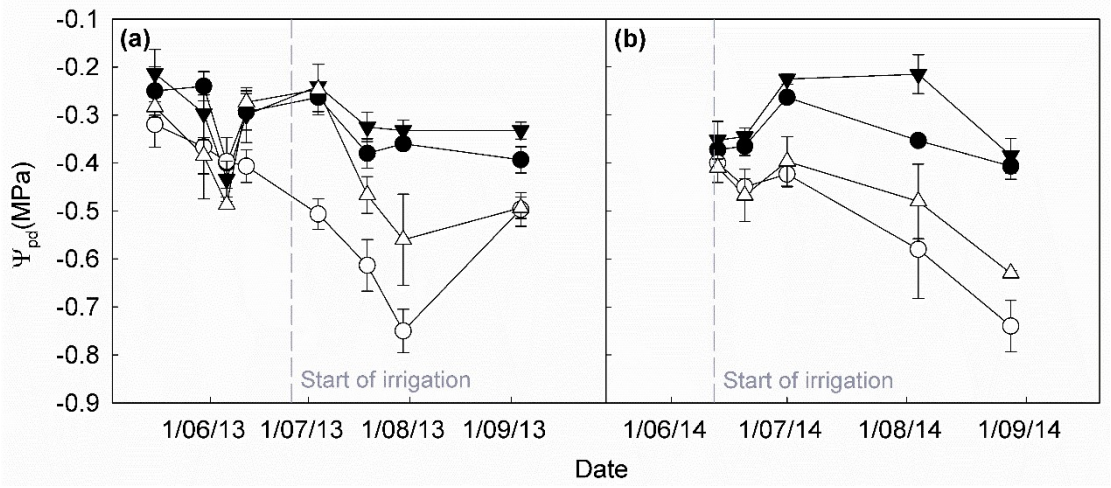

Figure 4. Predawn leaf water potential (Ψ_{pd}) measured in irrigated (●) and non-irrigated (○) Grenache vines, and in irrigated (▲) and non-irrigated (△) Tempranillo vines during (a) 2013 and (b) 2014 seasons. Bars indicate ± SE (n=4).

Spatial variation of soil respiration

Soil respiration fluxes were measured from five different locations in the vineyard at different phenological stages to determine spatial CO_2 dynamics in soil. Figure 5 shows soil CO_2 efflux measured in I and NI vines. During 2013 (Figure 5a), soil CO_2 efflux at positions along the vine row (1, 2 and 3) was significantly higher (60%) than positions between rows (4 and 5) in I vines. In NI vines, however, although soil respiration values were 14% higher in locations within the row than between rows, no significant differences were found among these positions. During 2014 (Figure 5b), these differences were less clear than those obtained in 2013, but again, the positions 1 and 3 were significantly higher (50%) than those measured in positions 4 and 5 in I vines. In NI vines, the differences between the positions 1 and 3 and the positions 4 and 5 were significant and those situated in the vine row were 25% higher than in those between rows. On average, the soil respiration in positions along the vine row were around 5.5 µmol CO_2 /m^2·s in I vines and around 3.2 µmol CO_2 /m^2·s in NI vines. Similar values between treatments were obtained in the positions between rows (3.5 µmol CO_2 /m^2·s) for I vines and (3 µmol CO_2 /m^2·s) for NI vines.

Combined analysis (Figure 5c) revealed that soil CO_2 efflux values were higher in position 1 and 3 in both treatments. Significant differences were obtained between the positions located along the vine row and the positions between rows in I vines. Nevertheless, for NI vines, significant differences were found between the positions

along the row and the position 5. In the context of irrigation, soil CO_2 efflux measured along the row was around 65% higher than those measured inter rows (position 5). In NI vines, however, only a 25% of increase was observed between both locations. Finally, it is worth noting that positions 4 and 5 (far from the wet bulb) in I vines showed similar values as in all positions of NI vines.

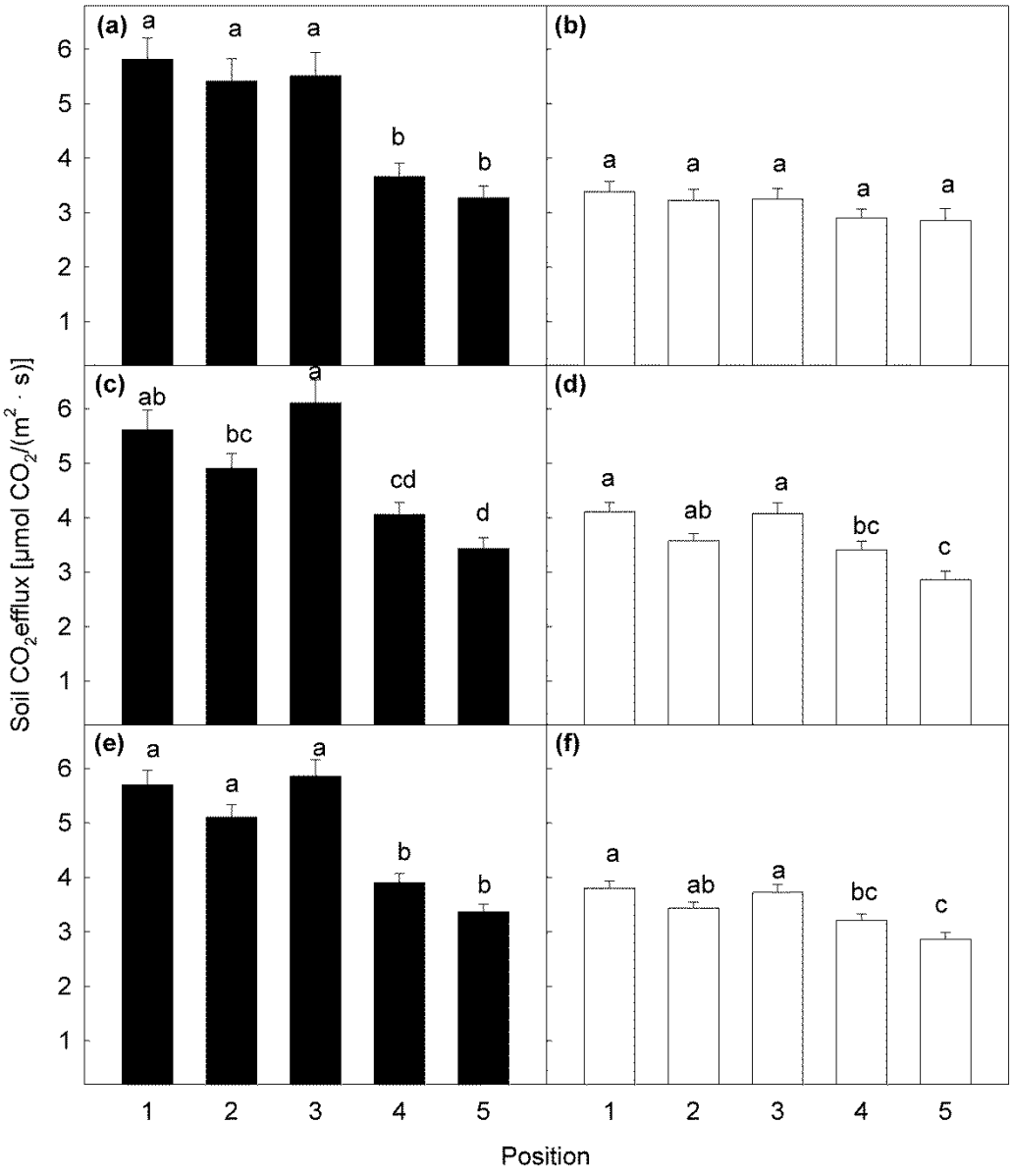

Figure 5. Mean soil CO_2 efflux from five different locations in (a, c, e) irrigated and (b, d, f) non-irrigated vines averaging the five phenological stages (budburst, flowering, pea-size, veraison, ripening and postharvest) during the seasons (a, b) 2013 (c, d) 2014, and (e, f) pooled data. Bars indicate ± SE (n=99). Different letters denote significantly differences according to Tukey´s test (P=0.05).

Table 3. Significance of the factors in a multiple regression model to predict soil CO2 efflux, including predawn leaf water potential, soil temperature and phenological stage.

	P-value (Significance)		
Factors	**Vine row**	**Inter rows**	**All data**
Ψ_{pd}	< 0.0001 ***	< 0.0001 ***	< 0.0001 ***
Soil temperatur	0.034 *	ns	ns
henological sta	< 0.0001 ***	ns	< 0.0001 ***

*, *P* < 0.1; **, *P* < 0.05; ***, *P* < 0.001. ψpd , predawn leaf water potential.

Table S1. Environmental variables registered during 2013 and 2014. Subtotal represents the sum of the values from April to October (vine vegetative period). Abbreviations: Avg. T = Average of mean temperatures; Max. Temperature = Average of maximum temperatures; Min. Temperature = Average of minimum temperatures; GDD = Growing degree days calculated with a base temperature of 10 ºC; RH = Relative humidity; ETo = Reference evapotranspiration rate; P = Precipitation; I = Irrigation.

	Month	**Avg. T (°C)**	**Max. T (°C)**	**Min. T (°C)**	**GDD**	**RH (%)**	**ETo (mm)**	**P (mm)**	**I (mm)**
	January	10.6	21.4	1.0	17.5	69.5	21.7	1.9	
	February	9.1	13.5	4.8	0.0	69.8	39.0	31.4	
	March	13.1	17.5	8.0	95.6	68.8	54.8	45.2	
	April	14.4	19.8	8.8	132.4	69.2	95.6	52.1	
	May	16.6	21.1	11.1	203.1	65.3	118.5	4.9	
	June	21.3	27.1	14.7	340.5	57.8	146.5	2.4	3.9
2013	July	26.1	32.4	19.3	499.3	55.8	168.2	0.6	26.7
	Augost	25.7	31.2	19.7	485.2	59.4	128.4	45.0	31.1
	September	22.2	27.7	17.3	367.0	70.9	97.1	24.1	13.4
	October	20.5	25.7	15.8	326.0	74.0	69.5	19.9	21.5
	November	13.4	17.0	9.6	100.8	72.7	35.9	139.2	
	December	10.6	15.5	6.3	17.6	79.9	16.9	29.3	
	Subtotal				2353.5		823.8	149.0	96.5
	January	11.5	15.2	7.6	46.3	80.0	26.6	44.5	
	February	11.5	15.8	6.7	40.7	76.4	47.3	26.7	
	March	12.3	17.0	7.4	71.6	67.1	78.2	18.8	
	April	16.2	21.5	10.2	185.1	69.1	104.4	60.0	
	May	17.9	22.6	12.3	243.0	64.9	116.8	34.0	
	June	23.0	28.6	16.9	390.7	58.0	136.4	20.4	20.1
2014	July	24.7	29.5	19.1	455.0	58.4	157.2	0.9	82.4
	Augost	25.1	30.1	20.2	469.5	68.9	134.2	8.7	54.9
	September	24.0	29.1	19.5	421.1	74.5	94.7	30.8	13.5
	October	19.9	26.0	14.9	308.4	76.9	70.3	8.3	
	November	15.4	19.9	11.3	161.1	83.8	37.8	126.1	
	December	11.1	14.9	7.2	33.1	79.2	23.3	87.6	
	Subtotal				2472.8		814.0	163.1	170.9

Soil respiration at different phenological stages

Soil respiration from positions 1 (in the vine row) and 5 (between rows) were compared to determine the influence of vine phenological stages on these two positions representing different Ra contribution (Figure 6). A repeated measures ANOVA analysis was performed to examine the differences among phenological stages, irrigations, cultivars and year effects at each phenological stage (budburst, flowering, pea size, veraison, ripening and postharvest). In general, a progressive rise in soil respiration at pea size was observed for position 1 followed by a decline during ripening and then recovery during postharvest. Soil respiration pattern at position 5 showed much lower values and a progressive decline from flowering until ripening with some recovery during postharvest.

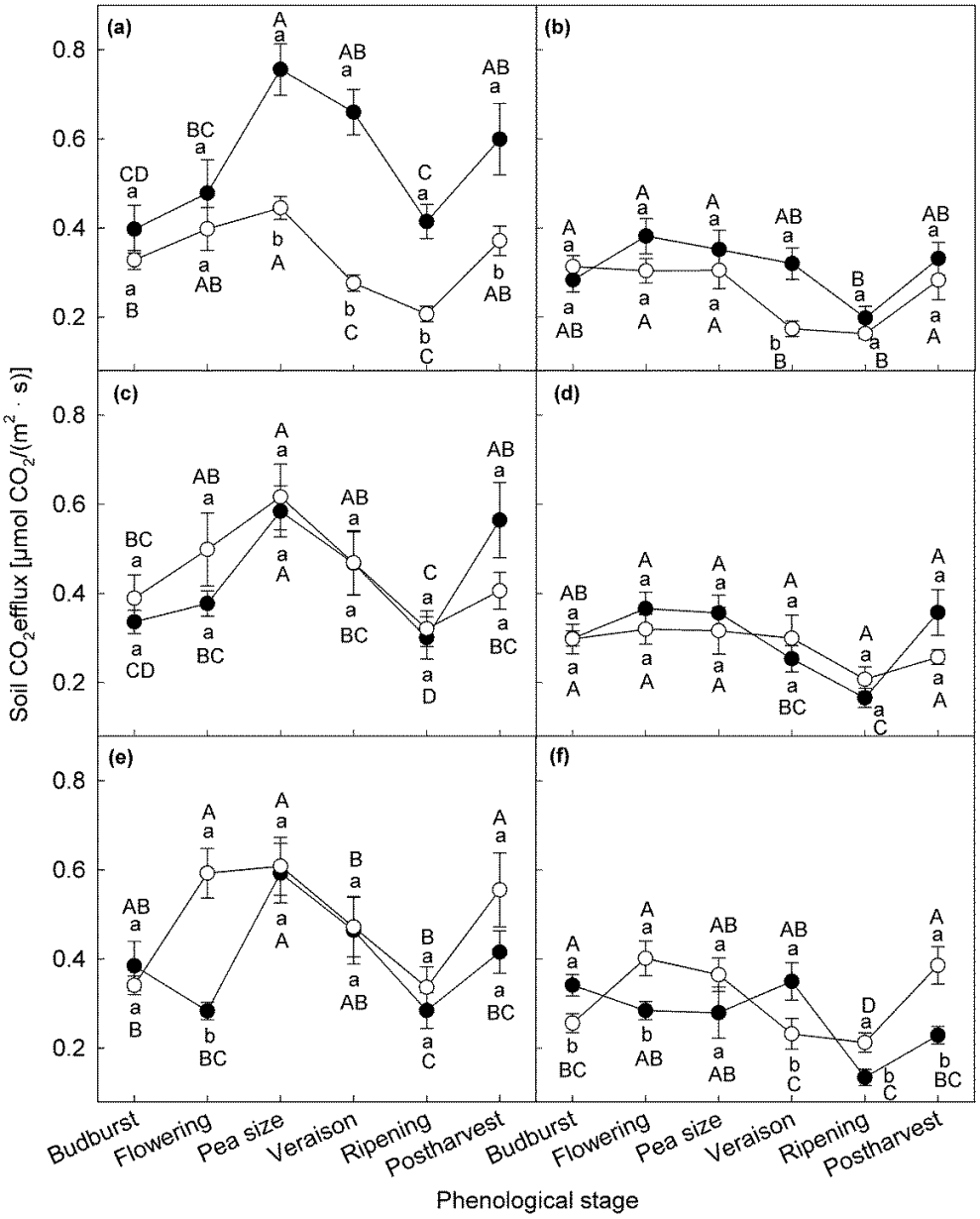

Figure 6. Soil respiration from (a, c, e) location 1 and (b, d, f) location 5 at several

phenological stages (a,b) in irrigated (●) and non-irrigated (○) vines; (c, d) in Grenache (●) and Tempranillo (○) cultivars; and (e, f) during 2013 (●) and 2014 (○) seasons. Bars indicate ± SE (*n*=6). Different lower case letters represent significant differences between irrigation treatments, cultivars and years at each phenological stage. Different upper case letters represent significant differences among phenological stages according to Tukey´s test (*P*=0.05).

In terms of water regime effects (Figure 6a, b), position 1 showed significant differences between treatments at pea size, veraison, ripening and postharvest stages., For position 5, however, there were no significant differences between treatments except at the veraison stage. Despite these differences between positions 1 and 5, the seasonal soil respiration pattern showed a gradual decline until ripening and then a rise during postharvest in both positions.

Figures 6c, d show the average soil respiration values for Grenache and Tempranillo measured at positions 1 and 5, respectively. Both cultivars showed a nearly identical seasonal trend in soil CO_2 efflux, however, the ANOVA and Pearson coefficients showed significant differences between seasons only at flowering (for position 1), ripening and postharvest (position 5) (Figure 6e,f). In general, 2013 season recorded lower soil respiration values than those in 2014.

In order to evaluate the soil water content, soil temperature and phenological stage effects on the soil CO_2 efflux, the data were subjected to a multifactorial regression analysis (Table 3). This analysis revealed that soil temperature had a significant effect only on in-row CO_2 flux. In contrast, the temperature did not have a significant effect on the inter-row efflux or on the combined data set (in-row and inter-row). Phenological stage showed a highly significant effect analysing all the combine data set and the vine row data. Pooled data analysis showed that the soil water content (expressed as Ψ_{pd}) had a significant effect on the respiration of within-vine row and between rows locations. Consequently, soil water availability and phenology were the most important factors in explaining the variations in soil respiration.

Discussion

Spatial variation in soil respiration

Soil respiration accounts for the largest fraction of crop carbon losses, thus its estimation is quite important for the integral carbon balances in the vineyard.

Accordingly, we compared genotype and irrigation effects on soil respiration in field-grown grapevines at several phenological stages. It is widely known that soil respiration is a function of the efflux of carbon into the rhizosphere, which in turn is influenced by soil microorganisms, soil moisture and temperature. These potentially confounding factors were separated by monitoring CO_2 efflux at different locations in the vineyard from drip-irrigated grapevines.

The spatial analysis of soil CO_2 efflux showed a dependence on soil moisture gradient. Moreover, in irrigated plants, soil respiration measured along the vine row (closer to the drip emitters) was significantly higher than between rows. Such a pattern was observed in both years with the irrigated vines wherein within the row soil respiration showed 50–60% increase over the respiration from inter-row locations. This spatial variation appeared to be in parallel with the large volume of wetted soil beneath the drippers promoting the production of fine roots and rhizosphere activity in such locations (Van Zyl 1984, Comas et al. 2005, Schreiner 2005). These values are higher than those reported by Lardo et al. (2015), which is most likely due to a sustained irrigation throughout the growing season in our study.

The difference in CO_2 efflux between the within-vine-row and inter-rows locations under NI conditions was not great; it was about 15–25%, comparatively much smaller than the difference found with I vines. These results reinforce the fact that soil respiration from within-vine-rows is greater than between rows, which is probably due to a greater root biomass and root density along the vine rows than between rows as previously reported in grapevines (Comas et al. 2005, Schreiner 2005, Franck et al. 2011). Based on this premise, we performed a separate analysis on the seasonal pattern of soil respiration from a representative within-vine-row location (more dependence on Ra) and a location from between rows (less dependence on Ra).

Soil respiration in relation to vine phenology

Vine phenology in our study influenced the seasonal pattern of soil respiration. The effect of irrigation, cultivar and year on soil respiration was analysed by taking into account vine phenology (Figure 6) at two locations differing in Ra. Soil respiration along the vine rows showed the influence of vine phenology and soil water availability besides soil temperature (Table 3). At the budburst stage, however, soil CO_2 flows were similar between I and NI vines, likely due to similarities in root activity and soil water

content in both treatments during this period (Figure 3). Some peaks in soil respiration were consistently observed; one at flowering, followed by pea-size stage, and a final peak during postharvest stage. Franck et al. (2011) observed three peaks of soil respiration during vine phenology and attributed such behavior to fine root production (budburst, middle-summer and leaf fall). Also, Van Zyl (1984) and Eissenstat et al. (2006) reported a peak of root growth at flowering and another peak at postharvest. In our study, average soil respiration values of I vines were higher at flowering stage even though plant water status was similar in both treatments (Figures 3,4) as the irrigation treatments had not yet started. This discrepancy possibly ensued from the cumulative irrigation effect in previous years resulting in higher root reserves, root development and below ground activity in the irrigated treatment. Irrigation was initiated at pea-size stage during which a second flush of shoot growth occurred in the irrigated vines (data not showed). Consequently, the difference between treatments was observed starting at the pea size stage at position 1 (Figure 6a). Irrigation also promoted higher stomatal conductance and photosynthesis (data not shown) that should result in a greater carbohydrate allocation to roots, larger root growth, and eventually an increase in rizosphere activity (Hopkins et al. 2013). Unequivocally, soil respiration measured at position 1 in I vines was higher than in NI vines, which was primarily associated with vine phenology and higher rates of photosynthesis and rizosphere activity (Ra).

In contrast, soil respiration measured at location 5 (Figure 6b) showed less dependence on vine phenology suggesting that it could be more linked to the heterotrophic component of soil respiration. Irrigated and NI vines showed similar soil CO_2 fluxes due to similarity in soil water content and the lower production of roots at this location. This phenomenon could be related to the lower root and rhizosphere activity during the summer ensuing from severe reduction in soil water content.

From veraison to ripening, a decrease in soil respiration rates was observed, which was expected as the developing fruit following fruitset becomes the largest sink for all the available photoassimilates to increase its biomass (Zamski and Schaffer 1996). Conversely, an increase in postharvest soil respiratory fluxes was observed in both treatments (Figure 6a) reflecting a postharvest flush of new roots (Schreiner 2005 Comas et al. 2010, Franck et al. 2011) and the loss of fruit competition during this period. Furthermore, it is well known that grapevines allocate resources to roots after harvest, which explains the lowest soil respiration rate before flowering and harvest

(Schreiner et al. 2005, Eissenstat et al. 2006). This is presumably due to little new root growth, as the developing leaves, shoots and bunches consume most of the carbon.

The soil CO_2 flows recorded during different phenological stages were similar between Grenache and Tempranillo (Figure 6b), hence, there was no significant effect of cultivar on soil respiration patterns. However, the effect of the year on soil respiration was quite evident at flowering, ripening and postharvest stages (Figure 6c). The significant difference between 2013 and 2014 at the flowering stage was probably due to a difference in amount of rainfall during the previous months (135.5 mm during 2013 and 184 mm during 2014). Moreover, in the 2014 season, the amount of water applied was higher than in the 2013 season, which would result in an increase in soil respiration fluxes once irrigation had started. Therefore, seasonal soil respiration appeared to be governed by vine phenology and the factors that influence the various growth stages. These include: temperature, soil water availability and carbon assimilation in spring; soil moisture limitations and fruit competition restricting root growth in summer; and moisture availability and carbohydrate supply after harvest (Comas et al. 2005, Eissenstat et al. 2006). Hence, it is possible to model soil respiration to gain a better understanding of vine phenology and physiology influenced by various environmental factors.

Soil water availability determines soil CO_2 efflux

Plant water status defined by Ψ determines stomatal conductance, CO_2 uptake by leaves (Flexas and Medrano 2002) and root production (Bauerle et al., 2008), but the relationship between Ψ_{pd} and soil respiration has received little attention. An important finding from this study was that soil CO_2 efflux depended on soil water content until a Ψ_{pd} value of -0.4 MPa was attained in NI vines (Figure 7, Ψ_{pd}< -0.4 MPa). I and NI vines differed in terms of Ψ_{pd} and soil respiration (Figure 7). Previous studies showed the effect of soil water availability and soil temperature on soil respiration [e.g. Scott-Denton et al. (2003), Davidson et al., 1998] whereas this study added another component (phenological stage), which explained carbon efflux patterns much better especially for along the vine-row locations. As per Schreiner (2005), however, soil water availability assessed by Ψ_{pd} was the determining factor for the locations between rows wherein the soil moisture declined more rapidly compared to within-vine-row causing a reduction in rhizosphere activity. Other below- and above-ground variables could also play an important role (Abramoff and Finzi 2014) such as photosynthesis

(Escalona et al. 2012b), consequently, it will affect carbohydrates availability, canopy development (Comas et al. 2005), root biomass (Franck et al. 2011) or the microbial activity (Schreiner 2005). Our study confirmed previous results about soil water availability affecting soil respiration and an adjustment of this effect caused by the plant growth pattern. Additionally, it suggested that soil respiration rate could serve as a gauge to assess root growth dynamics under field conditions.

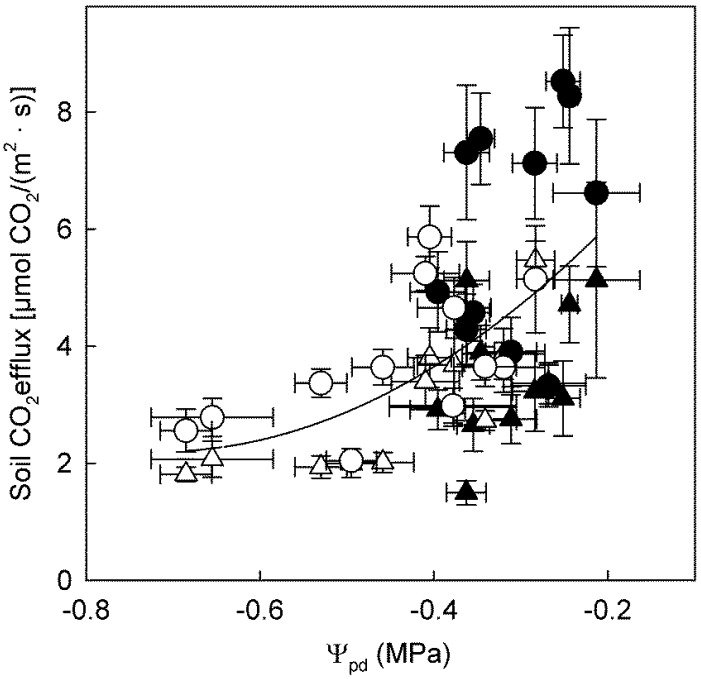

Figure 7. Relationship between predawn leaf water potential (Ψ_{pd}) and soil respiration ($r^2 = 0.36$, $y = 9.6 + 20.8x + 14.5x^2$) measured during the 2013 and 2014 seasons at all phenological stages in irrigated (●, ▲) and non-irrigated (○, Δ) Grenache (●, ○) and Tempranillo (▲, Δ) vines.

In conclusion, the spatial analysis of soil respiration in field conditions showed a high variability among locations wherein highest values occurred along the vine rows. Both soil water content and vine phenology regulated soil respiration by influencing water availability and probably, by increasing the availability of photo-assimilates in the roots. In general, a progressive increase in soil CO_2 efflux was maintained until pea-size stage followed by a progressive decrease until harvest, and a final peak during postharvest stage. The response of soil respiration to vine phenology as influenced by soil water availability was similar in both cultivars.

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CAPÍTULO 4

CHAPTER 4

Effect of genotype and plant water status on carbon balances and respiratory losses in grapevines (Vitis vinifera L.)

Effect of genotype and plant water status on carbon balances and respiratory losses in grapevines (*Vitis vinifera* L.)

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Abstract

Due to the important contribution of agriculture to the global CO_2 balance, new techniques are currently being developed to accurately estimate the carbon balance of different crops. Field studies to date in grapevines have been based on carbon allocation and biomass accumulation dynamics. Such field studies show the carbon balances whrere the respiratory losses and their changes along the vegetative cycle under different soil water availability are poorly considered or absent because of the paucity of information about them. In this work, we present the results of some measurements carried out during 2013 and 2014 to determine the effect of genotype and irrigation on respiratory rates and the plant carbon balance along the phenological cycle in field grown grapevines. The results show integrated carbon respiratory losses for leaves, fruits and roots along the vegetative cycle in irrigated and non-irrigated plants. Carbon respiration losses where higher for irrigated plants while the percentage of carbon loss respect to the total fixed carbon were higher in non-irrigated plants. An important, and significant genotype effect was present. Tempranillo recorded the highest values in carbon fixation, leaf and stem respiration, as well as the highest values in aerial biomass. However, Grenache obtained higher values of root respiration losses, and was the cultivar that located higher values of biomass of the permanent organs.

Introduction

Nowadays, the carbon balance of crops is being deeply studied to evaluate its contribution to carbon sequestration in order to develop adaptation and mitigation strategies that maximize the CO_2 sequestration and minimize the CO_2 emissions in a climate change scenario. The plant carbon balance integrates the CO_2 fluxes from photosynthesis and respiration, that result in the plant biomass accumulation. To determine plant carbon balance dynamics and the effect of genotype and environment, it is necessary to measure photosynthesis and respiration of the different plant organs, solving in some way important technical limitations.

In grapevine, there are many studies about the variations of photosynthesis throughout the phenological cycle of the plant (Escalona et al. 1999, De Souza et al. 2003, Schultz 2003, Baeza et al. 2005, Weyand and Schultz 2006) but the variability of respiration has not been so deeply studied. Some studies have developed models that integrate the respiratory component in these balances (Wermelinger et al. 1991, Poni et al. 2006, Weyand and Schultz 2006). Even so, there are few references in which the respiration of each organ is measured in order to obtain a more accurate carbon balance.

Due to the difficulties to accurately measure CO_2 exchange in field conditions, the carbon balance in vines has been determined from the accumulation of dry mass per year (Poni et al. 2006, Greer et al. 2011, Greer 2017) which in some case results in models to estimate overall above-ground non-permanent biomass (Vivin et al. 2002). Additionally, shoot biomass has been also measured in several cultivars (Miller et al. 1996, Greer and Sicard 2009). For permanent structures, we are aware only of the allometric models using vine age and trunk volume to estimate above-ground biomass. Lastly, as root mass is difficult to measure under field conditions, allometries for these organs are focused on establishing a ratio between root and trunk biomass. In a more recent contribution, different ways have been tested to estimate above-ground and below-ground total grapevine biomass using a range of cultivars, vine ages and environmental conditions (Miranda et al. 2017).

Few studies integrate CO_2 fluxes (photosynthesis and respiration) and biomass accumulation in order to complete the understanding of the plant carbon balance along the phenological cycle (Palliotti et al. 2004, Lakso et al. 2008). Escalona et al. (2012) calculated the amount of assimilates fixed by photosynthesis used in respiration (30-60%), and how the root is the organ that showed the highest expenses (75%) in potted and non-productive vines.

Due to the growing interest in knowing the carbon balance of the vine and the importance of the respiratory processes, a field trial was carried out during 2013 and 2014 to estimate both, the carbon assimilation and the carbon losses of field grown grapevines. In order to have a first insight on the variability of those respiratory losses, the field trial included two varieties (Grenache and Tempranillo) and two treatments (moderate irrigation and drought) measuring during two consecutive years (2013 and 2014). Respiratory losses were measured in leaves, stems, fruits and roots at different moments along the growing cycle. Thus, the present work aims to contribute to improve

our knowledge on the environmental and genetic induced variations of respiratory losses of field growing grapevines, under irrigation and drought. In this way, we study the effect of the genotype and the water regime on the respiratory component of the carbon balance of the vine.

Materials and methods

Plant material and treatments

The experiment was conducted in 2013 and 2014 seasons in a Grenache and Tempranillo experimental field at the University of the Balearic Islands. The vines were grafted on 110-Richter rootstocks and planted in 2009 in a NE-SW orientation and in a 1 m between plants and 2,5 m between rows planting pattern. The plants were trained to a bilateral cordon with 12 shoots per plant. The vineyard was implanted in a clay-loamy soil with 1.5 m of maximum depth. Two irrigation treatments were established on each cultivar: i) irrigation and ii) non-irrigation, consisted of withholding irrigation during the whole vegetative cycle. Weekly irrigation doses were calculated from the ETo registered by a meteorological station (Meteodata 3000, Geónica SA, Madrid, Spain) at the experimental site. The crop coefficient for the irrigation treatment (I) was first fixed at 30% of ETo recorded during 2013, and during 2014 the crop coefficient was increased to 40% of ETo in order to better differentiate the treatments. The irrigation period was from June to September both years using 3 drips per plant of 4 L per hour on a single pipe each row.

Predawn Leaf Water Potential

In this experiment, predawn leaf water potential (Ψ_{stem}) was measured every two weeks from budbreak to harvest, using a Scholander pressure chamber (Soil moisture Equipment Corp., Santa Barbara, CA, USA). The measurements were made around 1 hour before sunrise on four leaves per treatment, and the leaves were chosen from different plants in order to control the differences between treatments along the plant vegetative cycle.

Leaf net carbon assimilation rate

Leaf gas exchange measurements were performed with a portable gas exchange analyzer (Li-6400; Li-Cor Inc., Lincoln, Nebraska, USA). Environmental conditions in the chamber were >1000 µmol (saturation light), a CO_2 concentration of 400 µmol mol^{-1}

and ambient air temperature. Leaf gas exchange was measured at flowering, pea size, veraison, ripening and post-harvesting.

In order to measure leaf net carbon assimilation, five positions in canopy were selected according to Escalona et al. (2003): i) bottom east, ii) bottom west, iii) top east, iv) top west and v) inner canopy zone. The bottom positions (i and ii) are related to adult fully-expanded leaves, whereas top positions (iii and iv) are related to younger and/or expanding leaves. The inner canopy zone (v) is referred to leaves covered by at least one layer of leaves and shaded for most of the day except for occasional sun flecks. Measurements were done five times along the day in order to obtain the daily net photosynthesis of each type of leave.

Net daily carbon assimilation per plant was calculated from the net leaf carbon assimilation rate measurements and leaf area. Whole plant leaf area was calculated every two weeks, and the proportion of leaves from each leaf position was calculated by direct measurements following the methodology described by Sanchez-de-Miguel et al. (2011).

Night leaf respiration

Leaf night respiration was measured using the same equipment as leaf net carbon assimilation rate (Li-6400, Li-Cor) in two different types of leaves during the vine vegetative period: i) young expanding leaves, close to the apex and ii) adult leaves placed in mid-shoot in order to have rates referring to growth and maintenance, respectively. All measurements were performed in 4 plants per treatment and cultivar at night, between 23:00 h and 02:00 h, during budbreak, flowering, pea-size, veraison, ripening and postharvest.

At each phenological stage, the proportion of “growing” and “mature” leaf area was calculated. Total leaf respiration losses during the night were estimated by integrating the respiratory rates and the proportion of leaf area associated (growing and mature) at each phenological stage.

Stem respiration

Stem respiration rate was measured using a modified chamber which “embraces” the stem connected to the gas exchange analyzer Li-6400. Two different parts of the stem has been selected: i) apical zone and ii) mid to bottom zone, in order to

stablish the growth and maintenance respiratory losses, using a similar procedure than in leaves. All measurements were performed in 4 plants per treatment and cultivar at midmorning, during the phenological stages of flowering, pea-size, veraison, ripening and postharvest. The head of the IRGA was equipped with a light source (Li-6400-02B LED, Li-Cor) in order to measure under light and dark conditions to simulate the respiration during the day and night.

The shoot length and diameter was measured in 4 shoots per plant every two weeks. Moreover, the proportion of growing and mature shoots was estimated to integrate the respiratory rates and the length of shoot at each moment. The total respiratory losses from shoot respiration was calculated taking into account the number of shoots per plant.

Fruit respiration

Fruit respiration rate was measured using a home-made fruit chamber (described in chapter 2) connected to a gas exchange analyzer Li-6400. The entire cluster was measured on each of the three berry developmental stages: pea-size, veraison and ripening. These measurements were taken at midmorning using 4 plants per replicates (1 cluster per plant). The fresh and dry weight of each measured cluster was estimated by a non-destructive technique, using a linear regression between the volume and dry/fresh weight (described in chapter 2). All measurements were performed in 4 plants per treatment and cultivar at midmorning. In order to simulate the fruit respiration at night, the fruit chamber was covered with and isothermal sheet in each replicate.

The total respiratory losses from clusters was calculated taking into account the number of clusters per plant.

Root respiration

Root respiration was estimated from soil respiration measurements (Hernández-Montes et al., 2017; chapter 3). Soil respiration was measured using a CO2 flux chamber connected to a portable gas exchange analyzer (Li-6400, LI-COR, Lincoln, NE, USA). Measurements were performed in six stages, from bud break until harvest. Respiration was measured in three plants per treatment and five different positions per plant, in order to estimate the respiratory activity according to plant root system distribution. Total soil carbon losses per day and plant were calculated from the sum of

CO_2 efflux emission measured in the different positions multiplying by their respective soil surface and by the daytime. From that calculation, root respiration was estimated assuming that 52% of CO_2 soil emission is due to root respiration and the rest can be related to biological activity (mainly bacterial) and chemical soil (Franck et al., 2011). Seasonal integration of root respiration was calculated in each phenological stage to obtain the total carbon losses.

Biomass production

For each experiment (2013 and 2014), before the leaf fall, all plants were divided into three main organ types: stems (main and laterals), leaves (including petioles) and fruits (harvested according quality specifications). Plants parts were dried in an oven at 70 ºC until they reached a constant weight. During the winter 2014-2015, after the experiments were completed, the control vines were uprooted, with the root system carefully extracted from the soil. Roots were excavated as described in De Herralde et al. (2010). The bulk volume predefined by previous experiments was dug out with a small excavator equipped with a backhoe. Width within the row was half the plant distance to both sides (0.75 m) and the same distance towards the inter-row space. Depth was 0.6 m because of the presence of a plough hardpan. Then, borders of the soil hole were manually finished with the help of tools such as hoes and shovels. Below-ground trunk and main coarse roots were manually retrieved. The rest of soil plus roots was sieved with a battery of sieves of mesh size, ranging from 30 to 5mm, to retrieve as much roots as possible. All recovered roots were gently washed and wiped to eliminate soil particles. Then, trunk, cordons and roots were separated, oven-dried at 70°C for 10 days and weighed.

A specific carbon content was applied to the dry matter of each plant organ (Vivin et al. 2003) in order to obtain the grams of carbon produced per organ in a vine.

Results

Environmental conditions and plant water status

Environmental conditions were recorded during the experiment (2013 and 2014) showing data usually taken in Mallorca for these dates. Total rainfall from April to October (growing season) were149 mm and 163 mm, for 2013 and 2014 seasons, respectively, and the evaporative demand (ETo) was 823 mm and 814 mm, respectively

(Table 1). The growing degree days (GDD) accumulated from budbreak to harvest were 2354 and 2474ºC in 2013 and 2014, respectively (Table 1). In both years the mean daily temperature growing cycle of grapevines were compressed between 20 and 30 ºC having a maximum daily average per year of 32.4 °C in July 2013 and 30.1 °C in August 2014. Precipitation was almost null during these two months (0.6 mm, and 8.7 mm in 2013 and 2014, respectively). On an average, the irrigation applied during 2013 and 2014 was 95 mm and 177 mm. Initially, the crop coefficient for the irrigation treatment (I) was initially fixed at 30% of ETo in 2013, but in 2014 it was increased to 40% to distinguish better the treatments.

Table 1. Environmental variables registered during 2013 and 2014. Subtotal represents the sum of the values from April to October (vine vegetative period). Abbreviations: Avg. T = Average of mean temperatures; Max. Temperature = Average of maximum temperatures; Min. Temperature = Average of minimum temperatures; GDD = Growing degree days calculated with a base temperature of 10 ºC; RH = Relative humidity; ETo = Reference evapotranspiration rate; P = Precipitation; I = Irrigation.

	Month	Avg. T (°C)	Max. T (°C)	Min. T (°C)	GDD	RH (%)	ETo (mm)	P (mm)	I (mm)
	January	10.6	21.4	1.0	17.5	69.5	21.7	1.9	
	February	9.1	13.5	4.8	0.0	69.8	39.0	31.4	
	March	13.1	17.5	8.0	95.6	68.8	54.8	45.2	
	April	14.4	19.8	8.8	132.4	69.2	95.6	52.1	
	May	16.6	21.1	11.1	203.1	65.3	118.5	4.9	
	June	21.3	27.1	14.7	340.5	57.8	146.5	2.4	3.9
2013	July	26.1	32.4	19.3	499.3	55.8	168.2	0.6	26.7
	Augost	25.7	31.2	19.7	485.2	59.4	128.4	45.0	31.1
	September	22.2	27.7	17.3	367.0	70.9	97.1	24.1	13.4
	October	20.5	25.7	15.8	326.0	74.0	69.5	19.9	21.5
	November	13.4	17.0	9.6	100.8	72.7	35.9	139.2	
	December	10.6	15.5	6.3	17.6	79.9	16.9	29.3	
	Subtotal				2353.5		823.8	149.0	96.5
	January	11.5	15.2	7.6	46.3	80.0	26.6	44.5	
	February	11.5	15.8	6.7	40.7	76.4	47.3	26.7	
	March	12.3	17.0	7.4	71.6	67.1	78.2	18.8	
	April	16.2	21.5	10.2	185.1	69.1	104.4	60.0	
	May	17.9	22.6	12.3	243.0	64.9	116.8	34.0	
	June	23.0	28.6	16.9	390.7	58.0	136.4	20.4	20.1
2014	July	24.7	29.5	19.1	455.0	58.4	157.2	0.9	82.4
	Augost	25.1	30.1	20.2	469.5	68.9	134.2	8.7	54.9
	September	24.0	29.1	19.5	421.1	74.5	94.7	30.8	13.5
	October	19.9	26.0	14.9	308.4	76.9	70.3	8.3	
	November	15.4	19.9	11.3	161.1	83.8	37.8	126.1	
	December	11.1	14.9	7.2	33.1	79.2	23.3	87.6	
	Subtotal				2472.8		814.0	163.1	170.9

Predawn leaf water potential was measured in irrigated and non-irrigated plants in order to characterize the plant water status of irrigated and non-irrigated plants (Figure 1). Differences between irrigation treatments were found from the beginning of veraison (July) to harvest (September). Predawn leaf water potential of irrigated plants was maintained up to -0.4 MPa during the 2013 and 2014 growing seasons. For non-irrigated plants, there was a progressive decline in plant water status from veraison (around -0.5 MPa) to ripening (around -0.7 MPa) during 2013, and from veraison (around -0.4 MPa) to harvest (around -0.7 MPa) during 2014 in both cultivars.

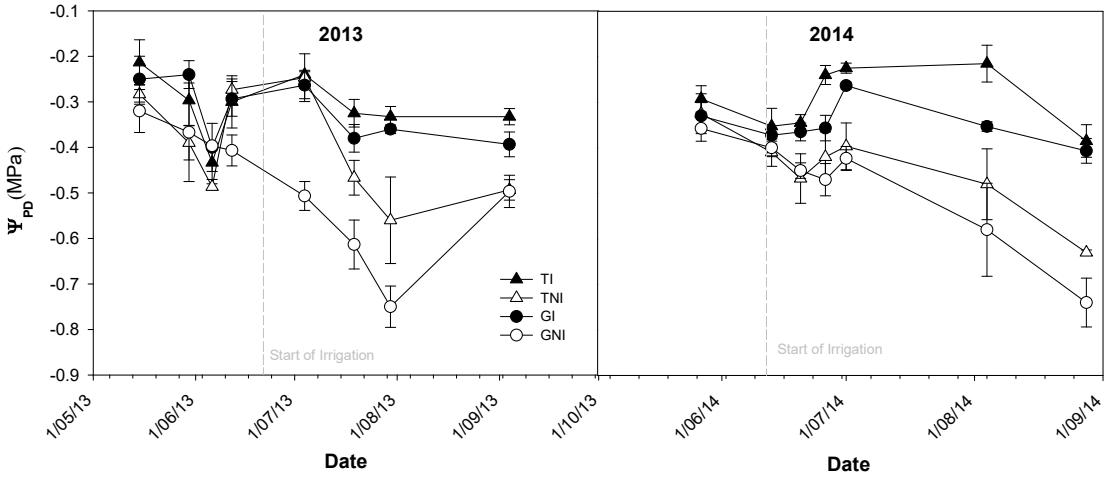

Figure 1. Predawn leaf water potential (Ψ_{pd}) measured in irrigated (●) and non-irrigated (○) Grenache vines, and in irrigated (▲) and non-irrigated (△) Tempranillo vines during (a) 2013 and (b) 2014 seasons. Bars indicate ± SE (n=4).

Net plant carbon assimilation by leaves

Net carbon assimilation of Grenache and Tempranillo leaves were estimated by integrating the photosynthetic rates from different types of leaves and recalculated for the whole plant leaf area at different moments of the phenological cycle (flowering, pea-size, veraison, ripening and postharvest). The integrative carbon assimilation values per plant and year are presented in Table 2. Tempranillo vines showed a clearly higher total carbon fixation per plant than Grenache ones during both, 2013 and 2014 seasons. In general, an increase of total carbon assimilation was observed in both cultivars in irrigated conditions, registering an increase carbon uptake of a 70% and 60% as a mean of 2013 and 2014 seasons in carbon fixation for Grenache and Tempranillo, respectively.

Table 2. Averages of final leaf area, total carbon fixation, leaf respiration (R_{leaf}), stem respiration (R_{stem}), fruit respiration (R_{fruit}) and root respiration (R_{root}) losses accumulated for irrigated (I) and non-irrigated (NI) Grenache (G) and Tempranillo (T) vines during the growing seasons 2013 and 2014. Values are means ± SE (n=4). Different letters denote significant differences (P<0.05) among cultivars and treatments in each year.

Year	Cultivar	Treat.	Leaf Area (m^2)	C fixation ($gC.year^{-1}$)	R_{leaf} ($gC.year^{-1}$)	R_{stem} ($gC.year^{-1}$)	R_{fruit} ($gC.year^{-1}$)	R_{root} ($gC.year^{-1}$)
2013	G	I	4.1±0.4 ab	2190.5±259.5 ab	148.6±12.6 bc	26.6±7.2 ab	202.6±45 b	581.8±47 ab
		NI	3.6±0.1 b	1238.2±106.4 b	104.2±4.6 c	9.8±0.8 b	201.2±21.4 b	419.8±20 c
	T	I	5.1±0.6 a	3435.2±734.4 a	264.1±24.3 a	52.8±9 a	358.5±51.5 a	567.6±38 a
		NI	4.1±0.3 ab	1915.5±280 b	210.9±4 ab	12.2±3.3 b	343.4±31.7 ab	443.2±43.5 bc
2014	G	I	6±0.4	2643.8±219.7 ab	211±21.3	40.8±7.7 b	515.5±148.4	594.1±29.6 a
		NI	4.3±0.2	1596±193 b	134±14.2	27.2±2.4 b	375.5±119.3	459.4±17.6 b
	T	I	6.7±0.4	3214.1±252.7 a	246.9±39.3	108.6±19.9 a	415.9±55.4	579.4±16.5 a
		NI	5.1±0.6	2212.8±449.5 ab	226.2±41.7	31.5±9.4 b	274.8±54.5	433.5±39.9 b
Cultivar			*	**	***	**	ns	ns
Treatment			**	***	**	***	ns	***
Cul*Treat			ns	ns	ns	***	ns	ns
Year			**	ns	ns	***	*	***
Year*Cul			ns	ns	ns	ns	*	ns
Year*Treat			ns	ns	ns	ns	ns	ns

Carbon losses due to respiration

The carbon losses from the respiration of leaves, shoots, fruits and roots expressed per plant and year basis are represented in Table 2. Total carbon losses derived from the different plant organs (leaves, stems, fruits and roots) during the vine vegetative cycle were calculated by integrating the measured respiratory rates during the vegetative cycle. Significant differences were found between cultivars in leaf and stems, having Tempranillo vines more losses of carbon due to respiration than Grenache. Also, significant differences between irrigation treatments were found in all the measured organs, having higher rates of respiration in well-watered conditions than in non-irrigated conditions. However, the effect of plant water status on respiratory losses was not equal among plant organs. Although the integrative carbon losses due to leaf respiration in non-irrigated plants was significantly lower than in irrigated plants, the maximum effect of irrigation was registered in root respiratory carbon losses. Consequently, the root respiratory component caused an increase in its proportion respect the total carbon fixed under the irrigation treatment. However, the rest of respiratory components did not show important changes under irrigation, in terms of the percentage respect to the total carbon fixed by photosynthesis.

The carbon losses of aerial organs (leaves, shoots and fruits) accounted to around 20-30 % from the total carbon fixed by photosynthesis. Total carbon losses derived from leaf respiration amounted to 1.7-1.8 kg C per plant in irrigated plants, and 0.7-0.8 kg C per plant in non-irrigated plants. These losses represented a 7-8% respect to the total carbon fixed by photosynthesis in irrigated plants, and 9-11% in non-irrigated plants (Figure 2). The carbon losses from the respiratory activity of stems were the lowest in relation to the rest of organs (Table 2). From the total carbon fixed by photosynthesis, only 1-2% was lost by stem respiration, although it represented on average 25% of the aerial biomass produced (Table 3).

The highest losses due to fruit respiratory activity were recorded in irrigated plants, both in Grenache and Tempranillo (Table 2). Although no significant differences were found between treatments in fruit respiration rates per dry weight unit, irrigated plants recorded higher fruit weight per plant than non-irrigated plants. These facts together made the integrated values of fruit respiratory carbon losses higher in irrigated plants than in non-irrigated ones. Even so, the respiratory losses respect to the total carbon fixed by photosynthesis of non-irrigated Grenache and Tempranillo plants were around 18%, and for irrigated ones about 13%.

The root was the organ with the highest respiratory cost in relation to the total carbon fixed by photosynthesis, presenting Grenache the highest percentages (25-30%).The carbon loss associated with root respiration represented 40-60% of the total losses. The results showed a higher carbon loss in the irrigation treatment (Table 2) likely due to the positive effect of moisture on root growth.

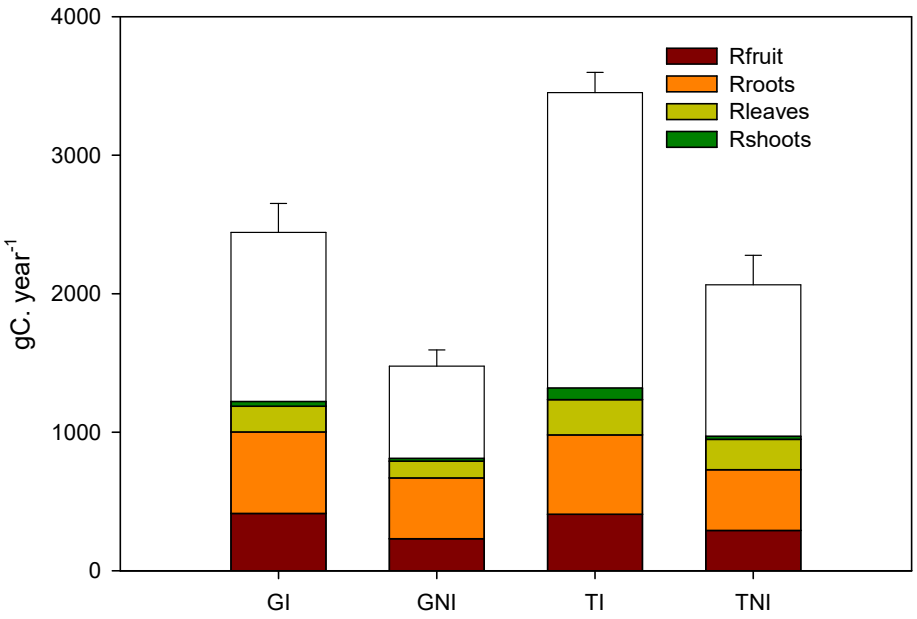

Figure 2. Total vine carbon fixed and respired (leaves, stems, fruits and roots) during the vegetative growth season for irrigated (I) and non-irrigated, Grenache (G) and Tempranillo (T) vines. (n=4).

Biomass production

Biomass production of irrigated and non-irrigated Grenache and Tempranillo plants is presented in Table 3. Water stress clearly affected the total biomass accumulated during the season, and the differences between cultivars and irrigation treatments in non-permanent and permanent organs were significant. On a dry weight basis, the proportion of leaf biomass, stems and fruits was around 18%, 25% and 50% respectively, in relation to the total aerial biomass produced during the year (Figure 3). Likewise, the proportion of leaf biomass, stems, fruits and permanent organs (arms, trunk and roots) was 12%, 15%, 27% and 46% respect to the total plant biomass, including permanent organs. The fruit was the most important carbon sink, and in this study represented around 50% of the total aerial biomass produced (Table 3). In general, Tempranillo presented higher values of biomass of non-permanent structures compared to Grenache. Also, water stress showed a clear reduction of biomass in both cultivars.

Table 3. Average of total biomass production from leaves, stems, clusters, roots, trunk and arms from irrigated (I) and non-irrigated (NI) Grenache (G) and Tempranillo (T) vines during 2013 and 2014 seasons. Values are means ± SE (n=4). Different letters denote significant differences (P<0.05) among cultivars and treatments in each year.

Year	Cultivar	Treat.	Leaves (g)	Shoots (g)	Clusters (g)	Total aereal organs (g)	Trunk and arms (g)	Roots (including main axis) (g)	Total permanent organs (g)
2013	G	I	367.8 ± 37 a	454.6 ± 53 b	828.1 ± 177 ab	1559.6 ± 88 bc	-	-	-
		NI	239.5 ± 11 b	302.8 ± 21 b	581.5 ± 58 b	1123.8 ± 70 c	-	-	-
	T	I	420.9 ± 49 a	690 ± 85 a	1462.5 ± 327 a	2573.4 ± 440 a	-	-	-
		NI	356.1 ± 37 ab	478.2 ± 80 ab	1357.7 ± 144ab	2192 ± 246 ab	-	-	-
2014	G	I	333.6 ± 37	480.8 ± 48	1322 ± 204	2469.9 ± 205 a	1098.9 ± 175	1096.4 ± 101	2195.3 ± 259
		NI	323.3 ± 61	461.7 ± 83	794 ± 179	1902.2 ± 103 ab	860.6 ± 64	830.4 ± 114	1691 ± 165
	T	I	469.2 ± 18	618.9 ± 63	1001.6 ± 64	2558.7 ± 88a	662 ± 25	1098.9 ± 65	1760.9 ± 85
		NI	297 ± 69	462.7 ± 99	716.9 ± 216	1721.9 ± 285 b	602.1 ± 17	849.7 ± 106	1451.8 ± 92
	Cultivar		**	**	*	**	**	ns	*
	Treatment		**	**	*	***	ns	*	*
	Cul*Treat		ns	ns	ns	ns	ns	ns	ns
	Year		ns	ns	ns	*	-	-	-
	Year*Cul		ns	ns	***	***	-	-	-
	Year*Treat		ns	ns	ns	ns	-	-	-

During 2014 the permanent structures of the plants were measured from the orchard in order to measure the biomass of those organs. Permanent organs (trunk, arms and roots) accounted for the major proportion of dry matter respect to the total of the plants, representing 55% and 45% in Grenache and Tempranillo, respectively. In this year, the total biomass of permanent structures was significantly higher in Grenache than in Tempranillo (around 1900 g dry mass/vine in Grenache and 1700 g dry mass/vine in Tempranillo). Those differences were mainly due to biomass of trunk and arms, that represented around 20% for Grenache, and around 30% for Tempranillo. Conversely, the biomass of aerial organs (leaves, shoots and fruits) was significantly higher in Tempranillo than in Grenache, accounting around 45% and 55% respect to the total vine biomass for Grenache and Tempranillo plants, respectively. On a dry weight basis, reproductive organs formed during the season accounted for 20-30% of the total biomass, and newly formed vegetative organs (leaves and shoots) represented a similar proportion.

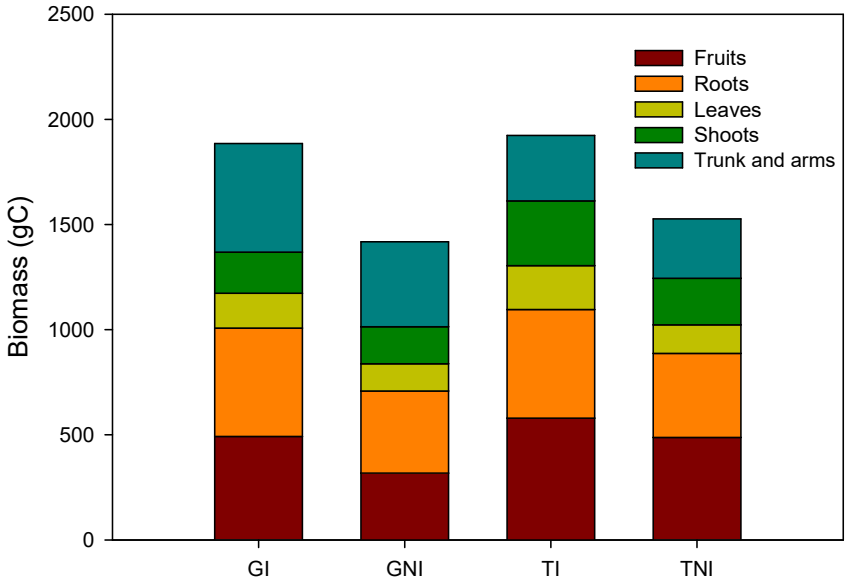

Figure 3. Allocation of vine biomass at harvest to leaves, stems, fruits, roots, trunk and arms of irrigated (I) and non-irrigated (NI) Grenache (G) and Tempranillo (T) vines.

Discussion

Biomass production is affected by carbon gain through photosynthesis but also by carbon losses through respiration (Valentini 2000, Griffis et al. 2004). However, the specific data about the respiratory components of carbon balance is still scarce in grapevines. The results presented in this study confirm the importance of respiratory processes on the carbon balance in field-grown grapevines, showing also the effect of genotype and plant water status that affect directly the inputs (photosynthesis) and outputs (respiration) of the vine carbon balance.

The data presented confirm a clear genotype effect on the components of the carbon balance. Firstly, the total carbon assimilation per vine showed differences between cultivars, probably due to the accumulated differences in the rates of photosynthesis (Bota et al. 2001, Escalona et al. 2012; Santesteban et al., 2009), leaf area, leaf structure and physiological behavior of the cultivars Grenache and Tempranillo (Tomás et al. 2014, Martorell et al. 2015). In this study, the complexity of a canopy conducted by a trellis system was represented by measuring leaves standing on five locations in the canopy (basal east oriented, basal west oriented, apical east oriented, apical west oriented and internal leaf) according to (Escalona et al. 2003). The integrated photosynthesis values all along the canopy and time clearly indicated that

Grenache and Tempranillo vines assimilated more than 1.8 kgC.year^{-1} and 2.5kgC.year^{1}, respectively. These values did not differ from those previously reported for vines in field conditions (Poni et al. 2006, Weyand and Schultz 2006).

Carbon losses due to the respiratory activity of leaves, stems, fruits and roots were estimated by integrating the respiration rates along the vine phenological cycle, taking into account the age of the organ and the phenological stage of the plant. Calculated total respiratory losses reached between 40 and 60% respect the total carbon assimilation (Table 2), according to the results in the potted experiment of Escalona et al. (2012). However, these values were about 10-15% lower than those ones obtained from whole-vine gas exchange measurements (Lakso et al. 1997, Poni et al. 2000) and agree well with calculated carbon requirements for vines when respiratory costs for growth and maintenance of other vegetative organs and fruit are deducted (Williams 1996). No significant differences were found between cultivars in terms of root respiratory carbon losses. However, significant differences between cultivars were found in root biomass during 2014 (Table 3). Franck et al. (2011) reported lower values of root respiration during the growing season using a trenching approach (no destructive) in a drip irrigated field experiment. In this study, the estimation of root respiration losses was calculated from the assumption that the root respiration accounts 52% from the soil respiration (Franck et al., 2011). However, the effect of irrigation on the root activity suggest that more studies are necessary about root respiration in field conditions.

Considering soil water availability, variations were induced by irrigation treatment. As expected, water stress reduced both the leaf area and photosynthesis. In consequence, non-irrigated plants fixed 30-40% less carbon than irrigated ones in both cultivars. Also, the respiration losses from leaves at night showed differences between irrigated and non-irrigated plants, with the irrigated Tempranillo plants reaching the greater losses, probably due to the higher leaf area and the higher respiratory rates of the growing leaves recorded in Tempranillo. The most important carbon loss was associated with root respiration, which represented 40-60% of the total losses.

The results show that carbon losses in whole plant respiration represent a significant part of total carbon balance in field-grown vines, as expected from previous references. However, the present work shows an important variation among the different organs of the plant and significant effects of the cultivar and soil water content

for most of those organs. Most of these effects were also significant for biomass accumulation. Among all plant organs, root respiration accounted for the largest fraction of total carbon loss in irrigated plants.

In summary, an important and significant genotype effect was present, opening the possibility of extrapolating the results to other genotypes with similar vegetative and physiological behavior. Tempranillo recorded the highest values in carbon fixation, leaf and stem respiration, as well as the highest values in aerial biomass. However, Grenache obtained higher values of root respiration losses, and was the cultivar that located higher values of biomass of the permanent organs. Therefore, the measurement of respiratory losses in different organs and treatments shows important variations which should be taken in consideration both to better understand carbon balances in grapevines as well as for a better assessment on the canopy leaf area, crop load and irrigation on carbon (and water) economy, and consequently, a more sustainable viticulture.

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DISCUSIÓN GENERAL

GENERAL DISCUSSION

DISCUSIÓN GENERAL

El capítulo de Resultados de esta Tesis se ha estructurado en cuatro apartados (1, 2, 3 y 4), y cada uno de ellos se corresponde con su artículo publicado o en proceso de publicación. Estos apartados se corresponden con los objetivos específicos citados en el capítulo correspondiente. Para abordarlos se llevaron a cabo diferentes experimentos enfocados a conocer con mayor detalle los procesos respiratorios en hojas (capítulo 1), frutos (capítulo 2) y raíces (capítulo 3) a lo largo del ciclo fenológico de la vid. Hasta ahora, gran parte de los estudios sobre respiración en vid se han llevado a cabo utilizando cámaras de planta entera (Perez Peña and Tarara 2004, Tarara et al. 2011), no permitiendo el estudio de cada órgano por separado. Otros estudios sobre respiración de diferentes órganos en vid se han realizado en plantas en maceta (Ollat and Gaudillère 2000, Poni et al. 2006, Escalona et al. 2012) debido a las dificultades técnicas que conlleva su media en campo. La experiencia previa del grupo en estudios ecofisiológicos en vid ha demostrado que las medidas en condiciones realistas de cultivo, aunque más complejas en su realización, siempre aportan conocimiento nuevo y más cercano a la realidad de la planta en sus condiciones de crecimiento habituales. En consecuencia, planteamos la investigación sobre los procesos respiratorios de los diferentes órganos de la planta en condiciones reales del cultivo de la vid. Además, este enfoque permite incorporar determinaciones de fotosíntesis en planta entera y valorar el peso de los procesos respiratorios en el balance de carbono total de la planta mediante la integración en el tiempo de la producción fotosintética y los costes respiratorios (capítulo 4). En el presente capítulo de discusión general, se aborda la discusión conjunta de los resultados de estos 4 capítulos a fin de ponderar las discusiones particulares de cada publicación y tratando de obtener una visión integrada de los procesos respiratorios en la vid en condiciones de campo.

1. Variación de la respiración de los diferentes órganos a lo largo del ciclo fenológico

Uno de los objetivos de esta Tesis fue estudiar el patrón de respiración a lo largo del ciclo fenológico de la planta. Desde brotación, la demanda de carbono aumenta debido a la formación de nuevas estructuras vegetativas y reproductivas que suponen un gasto elevado para la planta. Durante esta fase, las elevadas ratios de crecimiento en hojas y tallos se asocian a altas tasas de respiración (crecimiento y mantenimiento), tal y como se detalla en el capítulo de resultados (capítulo 1) de esta Tesis. En este apartado

se contrastó la respiración de crecimiento y mantenimiento de hojas en expansión y hojas maduras, mostrando que las hojas en los primeros estadios de crecimiento pueden llegar a respirar más del doble que hojas completamente maduras. Contribuciones anteriores ya dieron importancia a la ontegenia de la hoja a la hora de estudiar su respiración (Schultz 1991, Zufferey et al. 2000, Zufferey 2016). Por ello, el conocimiento de la edad de los diferentes órganos de la planta durante su etapa de crecimiento vegetativo es importante para calcular los gastos respiratorios de una manera precisa. Durante las primeras etapas de crecimiento vegetativo (brotación-floración), el coste respiratorio de las hojas y tallos fue creciendo a medida que el área foliar y la longitud de los pámpanos fueron aumentando, llegando a gastar 1gC por día entre hojas y tallos. Esto valores fueron bastante inferiores a los encontrados por Poni et al. (2006) en un estudio con plantas de Cabernet Sauvignon en maceta. En esta fase de alto gasto respiratorio de la parte aérea, las tasas de respiración radicular registran uno de sus mínimos durante el ciclo fenológico de la planta, tal y como muestra el estudio espacial y estacional de la respiración de suelo del capítulo 3 de esta Tesis y en coincidencia con los resultados de Franck et al. (2011). Posteriormente, desde los inicios de la floración, hay una fuerte competencia de los órganos reproductores por los fotoasimilados (Carmo Vasconcelos et al. 2009). Las flores tienen un alto coste respiratorio, y a la vez una importante capacidad fotosintética descrita en el caso de la vid por Lebon et al. (2005) y, más recientemente, por Sawicki et al. (2017). Desde este momento fenológico y hasta el envero, el crecimiento foliar sigue aumentando linealmente, lo cual sigue sumando los costes respiratorios de crecimiento y mantenimiento del dosel vegetal. A estos gastos hay que sumarle el alto coste respiratorio de las incipientes bayas, desde el cuajado hasta que finalizan su fase de crecimiento, debido a la respiración de crecimiento asociada a la multiplicación y expansión celular en los tejidos de las bayas. En el capítulo 2 de esta Tesis, las mayores tasas respiratorias por unidad de peso seco del fruto fueron registradas en las primeras fases de crecimiento (fruto verde y duro), en línea con los resultados obtenidos por Ollat and Gaudillère (2000) y Palliotti and Cartechini (2001). Así, durante este período se registraron las mayores pérdidas de carbono derivadas del fruto, llegando a 3 gC por planta y día. A su vez, la actividad respiratoria del suelo y la raíz sube desde floración para registrar su máximo antes de envero, tal y como se detalla en el capítulo 3 de esta Tesis, y en coincidencia con Franck et al. (2011) y Volder et al. (2005).

En los experimentos descritos en los capítulos 1, 2, 3 y 4 de esta Tesis, la parada de crecimiento vegetativo de las plantas se produjo en torno al estado fenológico de envero. La parada de crecimiento implica que todas las tasas de respiración de hojas y tallos están asociados al mantenimiento de los mismos. En este período del desarrollo, el dosel vegetal está formado en su totalidad, y la capacidad fotosintética de la planta es máxima. Al igual que la fotosíntesis y los costes respiratorios a nivel de planta están ligados directamente al área foliar, los procesos fisiológicos del desarrollo de la baya, también van a estar ligados al tamaño de la misma.

En el capítulo 2 de esta Tesis se muestra una relación entre el tamaño de baya y la respiración por unidad de peso seco. El coste respiratorio del fruto manifestó un incremento desde el cuajado hasta justo antes del inicio del envero, período en el que la baya conserva las propiedades fotosintéticas de sus tejidos (Breia et al. 2013). En este punto (baya verde-dura, Keller et al., 2010) la respiración asociada al crecimiento es muy baja, debido a que la baya ha finalizado la expansión celular. A ello hay que sumarle que, en ese momento todavía sigue habiendo una diferencia entre las medidas de respiración en luz y en oscuridad (capítulo 3), lo que reduce aún más los costes respiratorios derivados del fruto. Una vez finalizado el envero, el coste respiratorio por unidad de peso seco registró su mínimo en el ciclo de crecimiento de la baya, de acuerdo con los resultados de Ollat and Gaudillère (2000) y Poni et al. (2006). Sin embargo, los costes respiratorios integrados a nivel de planta incrementaron desde envero hasta la completa maduración. Esto puede ser debido en cierta manera a la pérdida de la capacidad fotosintética de los tejidos de las bayas. En este momento de máxima demanda del fruto por los fotoasimilados, la respiración radicular llega a su mínimo (capítulo 3). Tras la cosecha, la pérdida de la mayor competencia por los fotoasimilados, produce un repunte en la actividad radicular, asociada a la producción de raíces que han reportado algunos autores (Comas et al. 2005, Franck et al. 2011), con el consiguiente incremento de sus tasas de respiración.

Teniendo en cuenta el patrón de crecimiento vegetativo del dosel vegetal, la variabilidad que comporta (órganos en crecimiento y órganos adultos) a lo largo del ciclo fenológico, y las tasas fotosintéticas y respiratorias asociadas, se ha valorado también el balance de carbono integrado a nivel de planta. En este sentido, el capítulo 4 de esta Tesis describe los componentes respiratorios (hojas, tallos, frutos y raíces) respecto del total fijado por la fotosíntesis. La escasez de trabajos que integran la

asimilación de carbono, la respiración de distintos órganos, y la biomasa producida en condiciones de campo, hace que la información de este estudio sea valiosa para entender los procesos fisiológicos asociados a la práctica de determinadas prácticas agronómicas en viticultura.

Los datos recogidos en la presente tesis sobre la actividad respiratoria de los distintos órganos, se integró para cada uno de los muestreos realizados obteniendo una estimación de la respiración total de la planta en cada momento (Figura 1). Esta figura permite valorar la variación de los costes respiratorios a lo largo del ciclo y el peso de la respiración de cada órgano en cada momento. Así puede verse como los costes respiratorios de las hojas comenzaron representando un 15% de las pérdidas de carbono totales en brotación, para aumentar su peso a lo largo del ciclo vegetativo, manteniéndose en torno a un 30% de la respiración total de la planta, desde floración hasta que las bayas completaron su maduración. Sin embargo, el componente respiratorio asociado al tallo registró su mayor valor en floración (18%), para disminuir paulatinamente a medida que el tejido se fue lignificando (agostamiento) durante el ciclo vegetativo, hasta llegar a suponer un 3-4% de las pérdidas respiratorias totales en los estados fenológicos de maduración y postcosecha.

Por su parte el fruto supuso entre un 25% y un 40% de las pérdidas de carbono integradas totales de la planta durante el proceso de crecimiento de la baya. El momento en el que el componente respiratorio asociado al fruto fue más importante fue en maduración. En el capítulo 2 se describe como las mayores tasas respiratorias se registraron en las primeras fases de crecimiento de la baya. Sin embargo, durante la etapa de maduración de la baya, pese a registrar tasas respiratorias menores, la pérdida de la capacidad fotosintética del fruto y al aumento de tamaño de la baya le infirieron mayor peso (Figura 1).

De otra parte, la actividad respiratoria asociada a la raíz mantuvo un alto peso en el gasto total de la planta. Como se describe en el capítulo 3 de esta Tesis, el componente respiratorio de la raíz alcanzó su máximo peso en los momentos en los que la competencia del fruto no estaba presente (brotación, floración y postcosecha). Así, el componente respiratorio integrado asociado a la raíz registró el mayor porcentaje respecto al resto de componentes (hojas, tallos y frutos) en todos los estados fenológicos, excepto en el estado de maduración de la baya, donde la competencia del fruto llevó a mínimos la actividad radicular (Franck et al. (2011), 2011; capítulo 3).

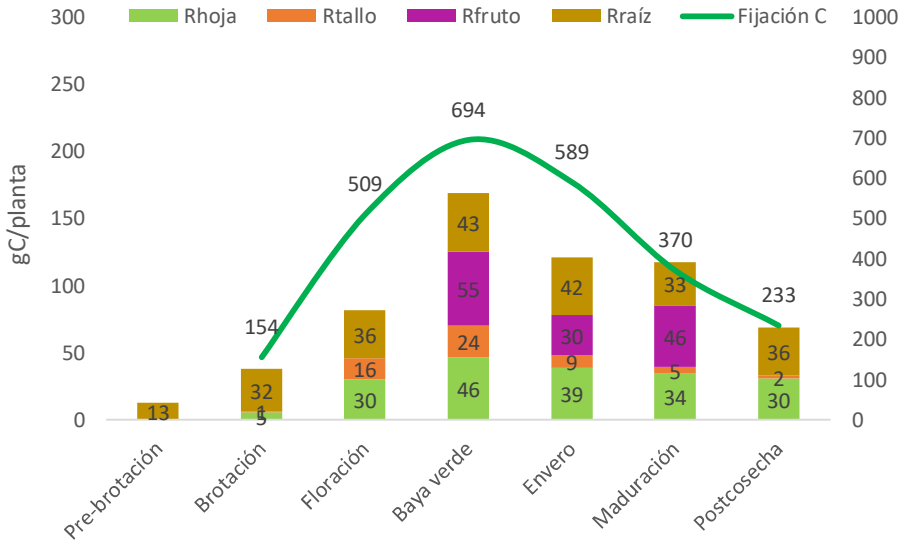

Figura 1. Evolución de la fijación y de las pérdidas de carbono integradas a lo largo de los diferentes estados fenológicos. Los números representan el valor medio (16 réplicas) del carbono fijado (línea verde) y del respirado por cada órgano.

2. Efecto del estado hídrico sobre la respiración

El riego es una de las técnicas agronómicas más extendidas en el manejo de un viñedo bajo las condiciones del clima mediterráneo. Por ello, el estudio de los efectos del riego y del estrés hídrico sobre los procesos fisiológicos adquiere gran relevancia. El efecto del estado hídrico de la planta sobre la fotosíntesis se ha estudiado en profundidad (Escalona et al. 1999, Flexas and Medrano 2002, Medrano et al. 2003, Collins et al. 2010, Tarara et al. 2011). Sin embargo, el efecto del estado hídrico sobre la respiración dispone de muchas menos referencias. Recientemente, da Silva et al. (2017) describen el efecto del estrés hídrico en la respiración de hoja en un experimento de plantas en maceta, demostrando que la respuesta puede implicar tanto una reducción en la demanda respiratoria, como una reducción en la producción de sustrato por la fotosíntesis. Los resultados obtenidos en esta Tesis, muestran sin embargo un efecto claro del estado hídrico de la planta sobre el flujo de CO_2 del suelo y la respiración radicular asociada durante la fenología de la planta (capítulo3), en línea con los resultados de Escalona et al. (2012). Sin embargo, el efecto del estado hídrico sobre las tasas respiratorias de cada uno de los órganos de la parte aérea no fue claro, en línea con los estudios encontrados hasta el momento en vid. Aun así, el análisis de las pérdidas integradas anuales sí que mostró diferencias entre las plantas regadas y en sequía

(capítulo 4). Estas diferencias tuvieron su origen por tanto no en las tasas respiratorias, sino en la diferencia que generó el estrés hídrico, en cuanto a área foliar, número y tamaño de bayas, así como en la producción de nuevas raíces y la actividad radicular.

La evolución de los componentes respiratorios a lo largo de la fenología de las plantas regadas y en sequía se detalla en la Figura 2. La proporción de cada componente respiratorio respecto del total respirado por la planta para cada estado fenológico se mantuvo para los dos tratamientos, excepto en el componente respiratorio del tallo. En este caso, las plantas regadas llegaron a registrar pérdidas de carbono derivadas de la actividad respiratoria del tallo alrededor de 20-25% en el estado de floración y 15% en la etapa de expansión de la baya. Estos costes se vieron reducidos a la mitad en las plantas no regadas en los mismos estados fenológicos.

Sin embargo, si referimos estas pérdidas respecto del carbono total fijado por la fotosíntesis, el porcentaje de carbono respirado por las hojas y por el fruto aumentó con la sequía en los estados fenológicos más avanzados, donde se registró un mayor estrés hídrico y una consecuente reducción de las tasas fotosintéticas y del área foliar.

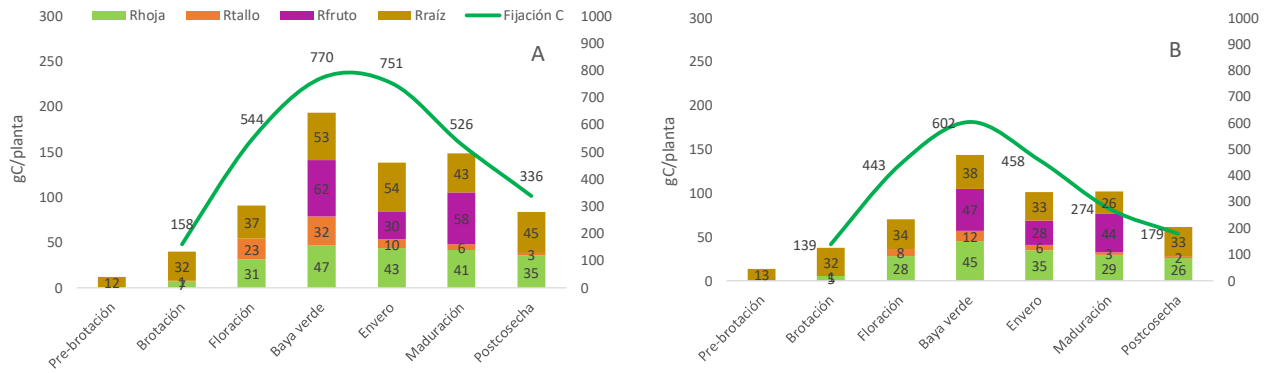

Figura 2. Evolución de la fijación y de las pérdidas de carbono integradas a lo largo de los diferentes estados fenológicos para los tratamientos de riego (A) y sequía (B). Los números representan el valor medio (8 réplicas) del carbono fijado (línea verde) y del respirado por cada órgano.

3. El efecto del genotipo sobre la respiración

La vid es una de las especies genéticamente más heterogéneas (Mulllins, M.G.; Bouquet, A; Williams 1992). Esta gran variabilidad genética ha permitido la adaptación del cultivo a una enorme diversidad de condiciones ambientales desde climas templados a tropicales. Esto supone una enorme plasticidad en las respuestas fisiológicas de la planta frente a condiciones adversas que se ha puesto de manifiesto en multitud de

publicaciones (Bota et al. 2001, Rogiers et al. 2004, Escalona et al. 2012, Pou et al. 2012, Tramontini et al. 2014). Sin embargo, apenas se dispone de información sobre el efecto del genotipo en la respiración de los distintos órganos. Los resultados de esta Tesis han demostrado un claro efecto del genotipo en las tasas respiratorias de los órganos vegetativos (capítulo 1) y reproductivos (capítulo 2) de la vid. En primer lugar, el capítulo 1 mostró las diferencias entre Garnacha y Tempranillo en las tasas respiratorias de hojas en expansión y maduras. Tempranillo invirtió más carbono que Garnacha para la formación de sus hojas durante la expansión de las mismas. Estas diferencias se vieron asociadas a características anatómicas y químicas en ambos cultivares, así como a un patrón de expansión característico para cada uno de ellos. Así mismo, el capítulo 2 refleja las diferencias entre Garnacha y Tempranillo en las tasas de respiración del fruto durante su ciclo de desarrollo. Nuevamente, Tempranillo registró los valores de respiración por unidad de peso seco más altos en todos los estados de crecimiento de la baya. De otra parte, las tasas respiratorias asociadas a la actividad radicular no mostraron un efecto del genotipo a partir del estudio espacial y temporal del flujo de CO_2 procedente del suelo (capítulo 3).

La evolución de fijación de carbono por las hojas y las pérdidas respiratorias medias de cada órgano para cada cultivar se muestran en la Figura 3. Los resultados medios integrados a partir de las tasas de fotosíntesis y el área foliar reflejaron como Tempranillo es capaz de fijar de media un 25% más carbono que Garnacha, diferencia que puede llegar hasta un 40% durante el envero. Las diferencias entre ambos cultivares se fueron incrementando a medida que el desarrollo vegetativo se fue completando. Así, Tempranillo perdió un 30% más carbono que Garnacha por la respiración nocturna de la hoja durante floración y un 40% a partir del proceso de desarrollo de la baya lo que parece asociarse a las mayores tasas respiratorias de crecimiento y a una mayor área foliar (respiración de mantenimiento) en Tempranillo. Las pérdidas por respiración de tallo en Tempranillo supusieron el doble de carbono que en Garnacha (Figura 3), en todos los estados fenológicos de la planta. El estudio de la integración de la respiración de fruto mostró como Garnacha registró mayores pérdidas de carbono únicamente en la última fase de maduración de la baya, probablemente debido al mayor rendimiento obtenido (kg/planta) en este cultivar, y a la pérdida de la capacidad fotosintética en este momento del desarrollo del fruto (capítulo 2).

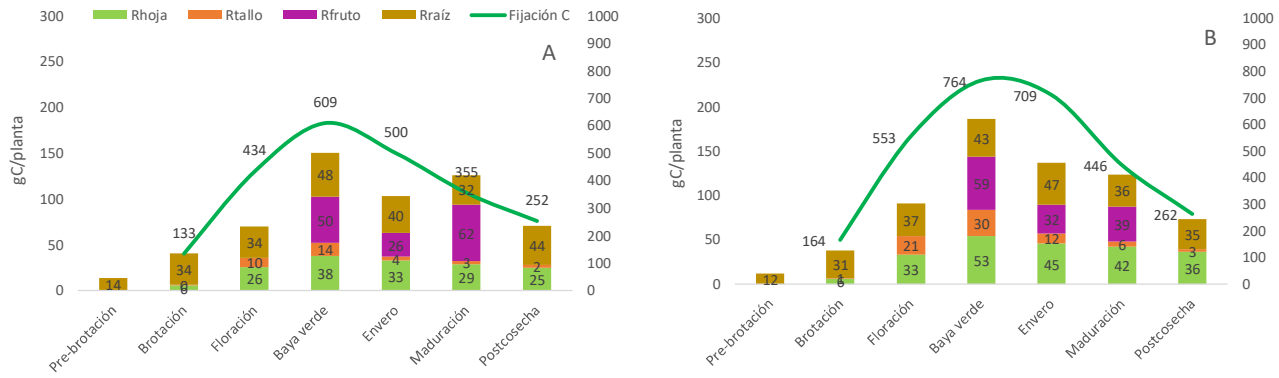

Figura 3. Evolución de la fijación y de las pérdidas de carbono integradas a lo largo de los diferentes estados fenológicos para los cultivares Garnacha (A) y Tempranillo (B). Los números representan el valor medio de 8 réplicas.

La producción de biomasa refleja el balance entre el carbono fijado por la fotosíntesis y las pérdidas de carbono debido a la respiración de los diferentes órganos. Por ello, en el capítulo 4 se llevó a cabo un estudio del balance de carbono y la biomasa producida en Garnacha y Tempranillo en condiciones de campo. El análisis de estos resultados puso de manifiesto que Tempranillo generó más biomasa en los órganos vegetativos anuales (hojas y tallos). Sin embargo, Garnacha acumuló mayor materia seca en los órganos permanentes de la planta (tronco y brazos). Estos resultados refuerzan la consideración previa de Garnacha como variedad más resistente a sequía, así como los resultados de anteriores estudios de nuestro grupo sobre estas dos mismas variedades (Tomás et al. 2014, Martorell et al. 2015). En estos estudios previos se describen las diferencias anatómicas, hidráulicas, hormonales y de eficiencia del uso del agua de Garnacha y Tempranillo coherentes con la reputación de Garnacha como variedad más adaptada a zonas semi-áridas. Por tanto, los resultados referidos en esta tesis refuerzan y amplían el papel de la base genética, reflejada en diferentes parámetros fisiológicos, en la adaptación de la vid a condiciones semi-áridas.

El conjunto de datos obtenidos sobre respiración, fotosíntesis, balance de carbono y producción de biomasa permite abordar la comparación de los balances obtenidos por medidas puntuales de fotosíntesis y respiración a lo largo del ciclo y la producción total. La relación entre estos valores se refleja en la figura 4. La biomasa de la parte aérea y los gastos respiratorios integrados de hojas, tallos y frutos se correlacionaron en una función lineal, con un coeficiente de regresión elevado (Figura 4A). Este coeficiente fue mayor que en las regresiones lineales derivadas de relacionar la producción de biomasa aérea con el carbono fijado (Figura 4B), o con el balance neto

de carbono de la parte aérea (diferencia entre el carbono fijado y el respirado, Figura 4C).

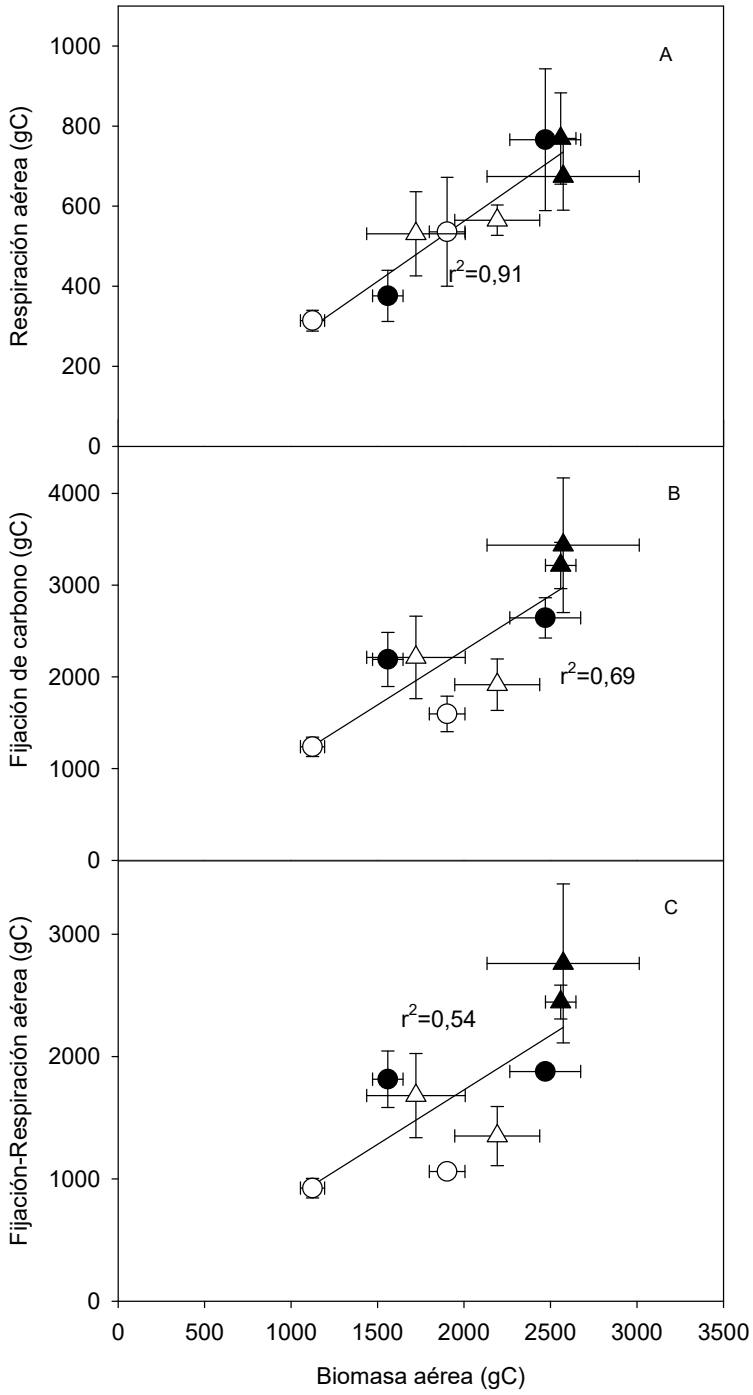

Figura 4. Relación entre la biomasa aérea anual (gC) con los gastos respiratorios aéreos anuales (A), con la fijación de carbono anual (B), y con la diferencia entre el fijado y el respirado (C) para los cultivares Garnacha (círculos) y Tempranillo (triángulos) en riego (negro) y sequía (blanco). Los valores son medias de 4 repeticiones.

Teniendo en cuenta la variabilidad intrínseca a los resultados en campo, las correspondencias obtenidas son muy altas y reflejan unas relaciones muy interesantes y de gran proyección para futuros estudios sobre la relación entre la producción primaria y el balance de carbono. Las diferencias en el grado de correlación pueden ser debidas a que, en las estimaciones integradas de las pérdidas respiratorias, la variabilidad es mucho menor que en el caso de las tasas fotosintéticas, como cabe esperar dada la fuerte variación en la intercepción de la luz en el dosel vegetal (Escalona et al. 2003, Medrano et al. 2012). Otros de los factores que influyen en la diferencia de correlación entre estas tres funciones lineales (Figura 4 A, B y C) podría ser también la translocación diferencial de fotoasimilados a la raíz o a los órganos permanentes, en la que se ha demostrado un efecto del genotipo.

Globalmente, los resultados y discusión de esta tesis muestran la respiración de la vid como una importante variable que es fuertemente dependiente del momento fisiológico de la planta, el estatus hídrico y el genotipo y además muestra características específicas que permiten caracterizar mejor el comportamiento de Garnacha frente a Tempranillo, en coherencia con resultados de tesis anteriores de este equipo de Investigación.

Estos resultados abren un amplio abanico de posibles aplicaciones a la gestión del viñedo, a la determinación de la contribución de la vid al secuestro de CO_2, a la determinación de los balances de carbono en planta entera en condiciones de campo y sobre todo a la consideración de la respiración como un proceso fisiológico clave para entender el funcionamiento de las plantas. Consecuentemente, tras este estudio inicial, es conveniente ampliar las determinaciones en diferentes cultivares, y prácticas agronómicas a fin de ampliar la base experimental del conocimiento sobre los procesos respiratorios y su peso en el balance de carbono en vid.

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CONCLUSIONES

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La hipótesis inicial de este trabajo fue que las tasas de respiración en vid presentan grandes variaciones inducidas por el ciclo fenológico de la planta, las condiciones medioambientales y el genotipo.
El objetivo general planteado fue precisamente la determinación de estas variaciones en condiciones de cultivo, identificando una serie de objetivos parciales que han articulado el trabajo experimental y el desarrollo final de la tesis.

Los resultados y discusión que preceden permiten concluir que globalmente hay una fuerte variación en las tasas de respiración y que dichas variaciones son significativas a efectos de conocer los balances reales de carbono en planta, así como para cualquier avance en los modelos de predicción del comportamiento, producción o cosecha en vid.

La discusión pormenorizada de los resultados (ver capítulos 1-4), así como la discusión general, permite establecer las siguientes conclusiones:

1. Las tasas de respiración de las hojas varían significativamente a lo largo de la expansión foliar, con caídas progresivas hasta los valores de la respiración de mantenimiento (hoja adulta).
2. Las tasas respiratorias de las hojas corresponden a variaciones en el patrón de expansión foliar y las características químicas, anatómicas y metabólicas de las hojas. Estas variaciones también reflejan un efecto marcado del genotipo en la respiración foliar.
3. La respiración ligada a la formación, crecimiento y maduración del fruto muestra un patrón de variación muy ligado al desarrollo del fruto. La respiración de fruto y su capacidad fotosintética fueron mayores en las primeras fases de desarrollo de la baya, con una bajada progresiva desde envero hasta la completa la maduración del fruto.
4. La respiración de racimos enteros es mayor que en bayas aisladas, lo que sugiere el importante papel del raquis a la hora de estimar la respiración de racimo a nivel de planta.
5. El genotipo marca significativamente las tasas de respiración asociadas al crecimiento, desarrollo y maduración del fruto.

6. La respiración del suelo presenta una alta variabilidad espacial y estacional alcanzando las mayores tasas de respiración las posiciones más cercanas a la planta.
7. La respiración de suelo estuvo afectada notablemente por el estado hídrico de la planta y la fenología de la vid. La respuesta de los flujos de respiración de suelo fue similar para los dos cultivares.
8. El balance de carbono en planta entera presenta notables variaciones con la fenología, el estado hídrico y el genotipo. El peso de estas variaciones hace necesario su consideración para cualquier modelo de crecimiento y producción del cultivo y sobre todo para valorar el efecto sumidero de carbono del viñedo.
9. El estado hídrico de la planta determina los balances de carbono por sus efectos tanto en las tasas de respiración como en el desarrollo vegetativo y reproductivo del cultivo.
10. El genotipo también afecta los componentes del balance de carbono, como consecuencia de la diferencia en las tasas fotosintéticas y respiratorias, en el área foliar y en el rendimiento de las plantas.
11. Tempranillo registró los mayores valores de fijación de carbono, de pérdidas respiratorias en hojas y tallos, así como los mayores valores de biomasa vegetativa anual. Garnacha, sin embargo, presenta mayores valores de acumulación de biomasa de los órganos permanentes de la planta.
12. La determinación de las tasas respiratorias en vid en condiciones de campo ha puesto de manifiesto que, como en otros caracteres fisiológicos fundamentales, los efectos de las variaciones ambientales y genotípicas sobre la respiración no pueden ignorarse cuando se trata de valorar los balances de carbono de este cultivo.