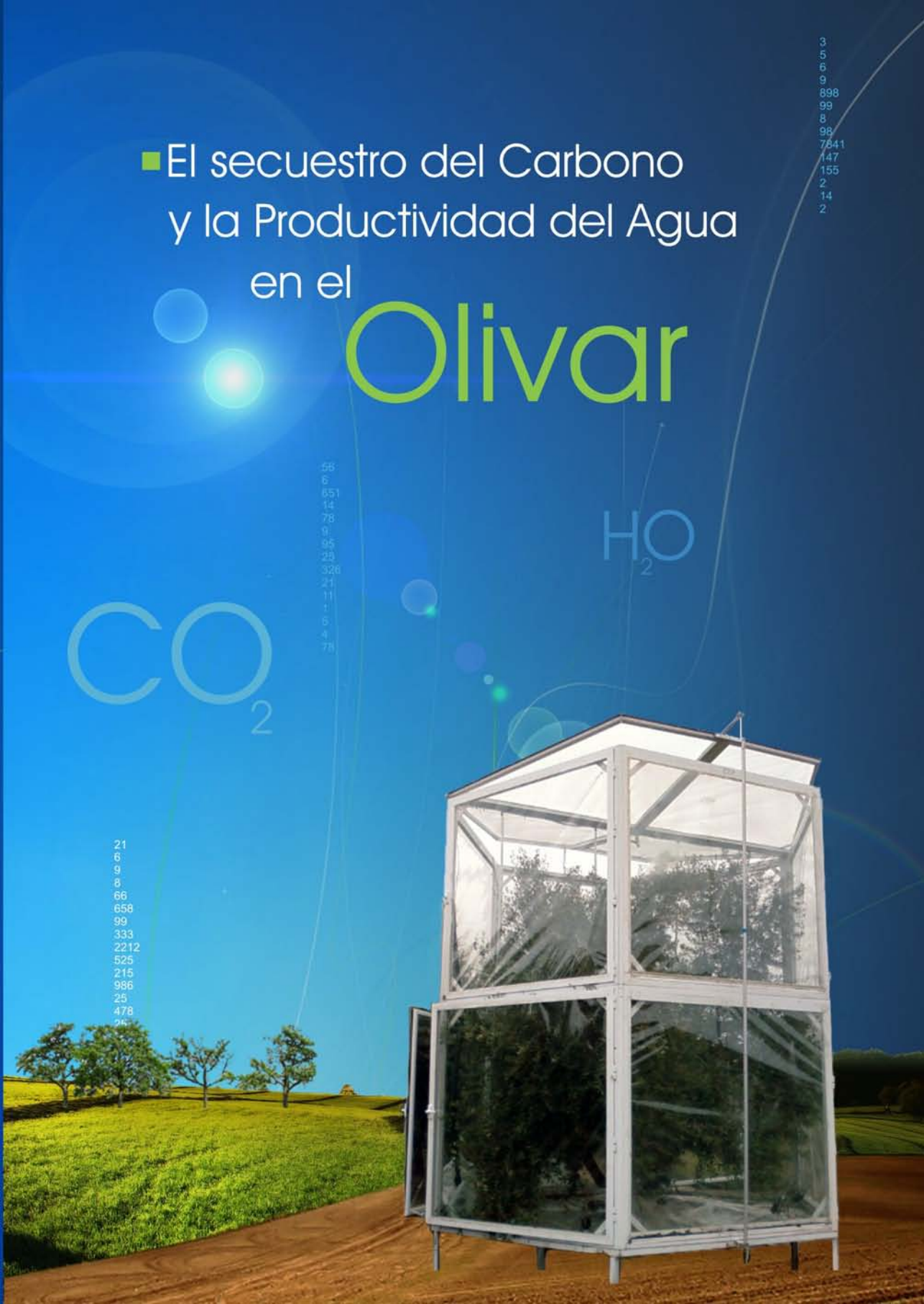
El secuestro del Carbono y la Productividad del Agua en el Olivar



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TESIS DOCTORAL

El Secuestro del Carbono y la Productividad del Agua en el Olivar

Carbon Sequestration and Water Productivity in Olive grove

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Three hundred trout are needed to support one man for a year. The trout, in turn, must consume 90,000 frogs, that must consume 27 million grasshoppers that live off of 1,000 tons of grass.

-- G. Tyler Miller, Jr., American Chemist (1971)

"All flesh is grass"

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List of symbols

θ	Leakage coefficient
a_1	Empirical coefficient
A_n	crown net carbon assimilation (net uptake of CO_2)
b	Empirical coefficient
C_a	CO_2 concentrations in the atmosphere
C_i	CO_2 concentrations in the sub-stomatal internal cavity
C_p	Specific heat capacity of air at constant pressure
C_s	Average CO_2 concentration at the leaf surface
D	Deficit pressure vapour
D_o	Empirical coefficient
E_{model}	Potential transpiration obtained by model
E	Transpiration

E_0	Optimised parameter
E_p	Potential transpiration
E_s	Evaporation from soil
ET	Evapotranspiration
ET_0	Reference evapotranspiration
F	Airflow
G_0	Canopy conductance when A is zero,
G_c	Canopy conductance
G_s	Stomatal conductance
K	Leaf hydraulic conductance
K_c	Empirical crop coefficient
P	Atmospheric pressure
Q_d	Daily fraction of intercepted PAR
T_0	Optimised parameter
T_a	Air temperature
V	Volume
γ	Psychometric constant
λ	Specific heat of vaporisation
ρ	Air density
ψ_l	Leaf water potential measurements
Γ	CO_2 compensation point,
ε	Relative error

List of abbreviations

CI	Control irrigation treatment
CR	Concentration regression method
GHGs	Greenhouse gases
GPP	Gross primary production (gross uptake of CO_2 due to photosynthesis)
IRGA	CO_2/H_2O Infrared gas analyser
LAD	Leaf Area Density
LMA	Leaf mass per unit leaf area
NEE	Net ecosystem exchange
NEP	Net ecosystem production
NPP	Net primary production
PAR	Photosynthetically active radiation
PLA	Plant leaf area
QR	Quadratic regression method
RDI	Regulated deficit irrigation treatment
R_a	Autotrophic respiration

R_{eco}	Ecosystem respiration
R_h	Heterotrophic respiration
RH_s	Relative humidity
R_m	Maintenance respiration
R_{plant}	Respiration of aboveground parts of the plant
R_{ref}	Reference respiration rate
r_s	Stomatal resistance
Rs	Global radiation
R_{sla}	Specific respiration rates per unit leaf area
R_{slm}	Specific respiration rates per unit leaf mass
R_{soil}	Soil respiration
R_{spm}	Specific respiration rates per unit plant mass
RUE	Radiation-use efficiency
VPD	Vapour pressure deficit
WUE	Water use efficiency

Resumen

El olivar constituye uno de los principales sistemas agrícolas en el área Mediterránea, y puede desempeñar un papel muy importante en el ciclo biogeoquímico como sumidero de gases de efecto invernadero (GEI). Sin embargo, la capacidad de fijar CO_2 en diferentes condiciones ambientales por parte de las cubiertas de olivar es casi desconocida. Por otra parte, la elevada productividad del agua en el olivar ha provocado que la práctica del riego en este cultivo se haya generalizado en los últimos años, llegando a convertirse en el más importante de la agricultura española de regadío, cuya sostenibilidad se ve amenazada de forma creciente por la escasez de recursos hídricos. Esta situación evidencia la necesidad de profundizar en el estudio del efecto del estrés hídrico en el intercambio de gases y en la productividad del agua en el olivar. Dada la dificultad de extrapolar resultados obtenidos a partir de medidas en hoja, sería de gran utilidad el disponer de herramientas que permitiesen realizar estas medidas a escala árbol en condiciones de campo.

Un método adecuado para medir el intercambio de gases en árboles individuales es el uso de cámaras. Sin embargo, hay una dificultad práctica en el diseño, construcción y operación de cámaras lo suficiente grandes como para incluir un árbol entero. El mayor reto de esta tesis fue el desarrollo de una herramienta capaz de medir el flujo de gases en árboles con volúmenes de copa de 20 a 30 m^3 en campo. Para ello se diseñó una cámara de cierre transitorio con ventanas y techos móviles para mantener la cubierta del árbol acoplada al ambiente mientras está abierta. Partiendo de unas condiciones naturales, mediante cierres transitorios se mide simultáneamente la variación en la concentración de CO_2 y vapor de agua por el intercambio de gases del árbol en el ambiente confinado por la cámara en un periodo de tiempo determinado. Los test realizados permitieron el desarrollo de un procedimiento estándar para medir el intercambio de gases en olivos.

Para caracterizar las variaciones diurnas y estacionales de las tasas de fotosíntesis a nivel de cubierta y el efecto del estrés hídrico en el intercambio de carbono y vapor de agua, se midió la asimilación neta de CO_2 (A_n), la transpiración (E) y la eficiencia en la transpiración (WUE) de olivos adultos en un periodo de 2 años en una parcela de olivar en Córdoba (España) con dos tratamientos de riego: control sin estrés hídrico (CI) y riego deficitario controlado (RDI). Independientemente del tratamiento de riego, la evolución de la conductancia estomática a lo largo del día presentó los valores máximos por la mañana temprano (8:00-9:00 GMT), disminuyendo de forma gradual desde ese momento hasta la puesta de sol. Mientras que la asimilación neta mostró un patrón similar al de la conductancia, la transpiración alcanzó sus valores máximos a primera hora de la tarde, como resultado del efecto conjunto de la conductancia de la cubierta y la demanda evaporativa (DPV). La asimilación neta (A_n) diaria varió estacionalmente con valores extremos de 9.6 g CO_2 m^{-2} d^{-1} en diciembre de 2006 y 22 g CO_2 m^{-2} d^{-1} en septiembre de 2007, para el tratamiento CI. El estrés hídrico redujo significativamente la A_n diaria en el tratamiento RDI. La WUE instantánea varió de forma dramática desde valores de 30 g CO_2 L^{-1} a primera hora del día hasta 2.7 g CO_2 L^{-1} al final de la tarde, siguiendo la típica relación inversa con el DPV.

Aunque las diferencias en los valores instantáneos de WUE entre los dos tratamientos de riego fueron muy pequeñas, el déficit hídrico provocó un aumento notable en la WUE diaria, como resultado de un mayor control estomático en los periodos del día con mayor demanda evaporativa (DPV). Las medidas permitieron la calibración de un modelo de conductancia basado en la relación conductancia-asimilación que contribuyó a evaluar el efecto del estrés hídrico en la WUE. La mejora de la WUE en el RDI sugiere el uso de estrategias de riego deficitario como método eficiente para maximizar la acumulación de biomasa y la productividad en un escenario de escasez de agua cuando el estrés hídrico se concentra en el verano (VPD alto). Sin embargo, los límites de déficit hídrico para evitar efectos secundarios negativos (reducción en el índice de cosecha, la senescencia foliar, etc.) deben ser definidos.

En último lugar, se presentan las primeras medidas de respiración a nivel de árbol en un estudio centrado en comprender cómo los patrones de respiración de la planta y el suelo de una parcela de olivar regulan la capacidad para el secuestro de carbono y cómo la respiración de los árboles responden a la temperatura, fenología y composición específica de órganos de la planta. Los resultados obtenidos en una serie de experimentos indicaron que; la respiración del suelo fue un componente importante de la respiración a nivel de ecosistema a pesar de que estuvo fuertemente limitada en el suelo seco. La respiración de raíces, micorrizas, y organismos descomponedores (respiración heterótrofa) en suelos permanece desconocida y requieren ser estudiadas. Mientras que la respiración del suelo fue constante, la respiración de la planta varió de forma proporcional a la temperatura. Este patrón hizo que la respiración del suelo fuera el mayor contribuyente de la respiración a nivel de ecosistema cuando la temperatura del aire fue inferior a 20 ºC. Los componentes de mantenimiento y crecimiento de la respiración fueron determinados en un experimento con olivos jóvenes sometidos a periodos prolongados de oscuridad y podas selectivas. Aproximadamente el 30% de la respiración de las plantas fue asignada a su componente de crecimiento y el 70% a la de mantenimiento, cuya dependencia con la temperatura fue determinada empíricamente para sus diferentes órganos. El coeficiente específico de mantenimiento (la tasa de respiración por unidad de masa de un órgano determinado a una temperatura de referencia) medido en frutos fue similar al de hojas, siendo para ramas el más bajo. En experimentos similares con árboles adultos, la respiración del tronco representó el 6% de la respiración total de la planta. Los resultados obtenidos indicaron que generalmente la respiración específica de un árbol está más relacionada a su masa foliar que a su masa total o su área foliar ya que las hojas fueron los principales contribuyentes a la respiración de las planta. El coste de mantenimiento tan reducido encontrado en madera estructural indica que la respiración de la cubierta se ve afectada mínimamente por diferencias en la biomasa leñosa.

Sumary

Olive groves form one of the main agricultural systems in the Mediterranean area, and may play an important role in the biogeochemical cycle as a sink of green-house gases (GHGs). Nevertheless, the capacity of olive canopies for carbon fixation under different environmental conditions is almost unknown. Moreover, the high water productivity of the olive has favoured the practice of irrigation, becoming the most important irrigated crop in Spain. In this context, the sustainability of irrigated olive orchards is threatened by the scarcity of water resources. Further studies of the effect of water stress on gas exchange and water productivity in olive orchards, going beyond leaf level measurements, would be very useful to improve the efficiency of water use and deficit irrigation practices.

A suitable method for measuring gas exchange of individual trees is the use of large canopy chambers. Nevertheless, there are practical difficulties in the design, construction and operation of chambers large enough to enclose a whole tree. A goal of this work was the development of a technique to measure canopy gas exchange of single trees in the field using a transient-state chamber. The chamber design allows enclosing a medium-size orchard tree with chamber top and windows that can be left open, imposing minimum disturbance to the tree environment. Transitory closures are performed to measure simultaneously the CO_2 exchange and transpiration of the enclosed trees. We demonstrate that it is possible to measure the CO_2 and water vapour exchange rates of tree crowns up to 20-30 m^3 with a chamber of the transient-state type. The tests performed the development of a standard procedure to measure the gas exchange of olive trees.

To characterise the diurnal and seasonal variations of canopy photosynthesis and the effect of water supply on the carbon and water exchange of olive trees, the canopy assimilation (A_n), transpiration (E) and water use efficiency (WUE) of mature olive

trees were investigated in a 2-year study in an olive orchard in Córdoba (Spain) with different irrigation treatments: control with no water stress (CI) and regulated deficit irrigation (RDI). Regardless of irrigation treatment, changes in stomatal conductance throughout the day showed the maximum values in the early morning (8:00-9:00 GMT), decreasing steadily from that time until sunset. While the net assimilation (A_n) rate showed a similar pattern as that of conductance, transpiration reached their peak in the early afternoon, as a result of the combined effect of canopy conductance and the vapour pressure deficit (VPD). Daily A_n varied seasonally with values ranging from 9.6 g CO_2 m^{-2} d^{-1} in December 2006 to 22 g CO_2 m^{-2} d^{-1} in September 2007 for the control treatment. Water stress significantly reduced the daily A_n of the RDI treatment. The instantaneous WUE varied dramatically from values of 30 g CO_2 L^{-1} in the early morning down to 2.7 g CO_2 L^{-1} g CO_2 L^{-1} at sunset, following the typical inverse relationship with the VPD.

Although differences in the instantaneous values of WUE between the two irrigation treatments were very small, the water deficit caused a marked increase in the daily WUE as a result of increased stomatal control at times of the day with greater evaporative demand (VPD). The measurements allowed the calibration of a conductance model based on the relationship conductance-assimilation which helped to assess the effect of water stress on WUE. Improving WUE in the RDI suggests the use of deficit irrigation strategies as an efficient method to maximize the accumulation of biomass and productivity in a scenario of water scarcity when water stress is concentrated in summer (high VPD). However, water deficit limits to avoid negative side-effects (reduction in harvest index, leaf senescence, etc.) must be defined.

Finally, we present the first tree-level measurements of respiration in large olive trees and additional results to understand how plant and soil respiration patterns regulate the capacity of olive orchards for carbon sequestration and how respiration of olive trees responds to temperature, phenology and organ plant composition. The results indicated

that soil respiration was a large component of ecosystem respiration and was strongly limited in dry soil. Nevertheless, respiratory activities of plant roots, of the mycorrhizal fungi and decomposers organism (heterotrophs) in soils remain uncertain and require further studies.While soil respiration was almost constant, plant respiration varied in proportion to temperature. Soil respiration was the largest contributor to ecosystem respiration when air temperature was lower than 20ºC. Maintenance and growth components of aboveground plant respiration were determined in young trees using prolonged darkness periods and selective pruning. Approximately 30% of plant respiration was growth and 70% maintenance respiration, whose dependence with temperature was empirically determined for different organs. The maintenance coefficient in fruits was closed to that found in leaves, while the coefficient for branches was the lowest. In adult trees, respiration of the trunk accounted for 6 % of the total aboveground plant respiration. Plant respiration was more closely related to leaf mass than plant mass or leaf area. Therefore, leaves were the main contributors to above-ground plant respiration. The small maintenance cost of structural wood indicates that aboveground plant respiration is weakly affected by differences in woody biomass.

General introduction

Olive (*Olea europaea* L.) is one of the most significant agricultural systems in the Mediterranean area, and may play an important role in the biogeochemical cycle as sink of greenhouse gases (GHGs). However, little information is available on the capacity of olive orchards for carbon sequestration (Villalobos et al., 2006). The net carbon assimilation is strictly dependent on the balance between photosynthesis (carbon gain) and respiration (carbon release). For reliable estimations at the stand level information about the magnitude of crown net assimilation and respiration in photosynthetic and non-photosynthetic plant organs is required. However, those measurements are complicated to perform in the field and the scaling procedures are not always straightforward. A better knowledge of the dynamics of carbon exchange in olive canopies under different environmental and management conditions is essential to develop a simulation model for quantifying yield and carbon accumulation.

Spain is the main world's producer of olive oil with more than 2.5 Mha of cultivated area. Rainfed olive orchards which are characterized mainly by low plantation density and variable yields are being replaced by new intensive irrigated ones. Olive has recently become the main irrigated crop in Spain, covering more than 400 000 ha. Water scarcity prevents most irrigated olive orchards from meeting the crop water requirement, thus demanding the development of deficit irrigation strategies (Iniesta et al., 2009).

One of the most promising deficit irrigation strategies is the *Regulated Deficit Irrigation* (RDI) which consists of reducing water supply during phenological periods when the impact of water stress on yield is minimum (Chalmers et al., 1981). The work of Iniesta et al. (2009) and similar studies (e.g. Girona et al., 2002; Moriana et al., 2003) have shown that deficit irrigation improves the water productivity (yield/water applied), although the main causes of this improvement are still not clear. The water use efficiency (WUE) - defined as the ratio of carbon assimilated per unit of transpired

water - is a key parameter in crop simulation models that evaluate optimum water use by analyzing the response of plants to water stress. Besides, WUE is not only essential to improve irrigation management but also to quantify biomass, water use, productivity and carbon exchange of olive ecosystems under different climatic and management scenarios.

Components of the Carbon Balance

The carbon pools defined by the Kyoto Protocol are aboveground biomass, belowground biomass, litter, soil carbon and wood residues (IPCC, 2007). Estimations of the carbon pools are being performed with three main methodologies; inventories of pool constituents, direct flux measurements and models. The quantification of carbon fluxes is a fundamental requirement to estimate the evolution of carbon pools and to address the dynamics and responses of the carbon cycle to environmental drivers. The research concerning climate change uses models of global and ecosystem carbon cycle to predict the impact of climate on the carbon pools dynamics of the world biomes. Besides, world agriculture is facing the challenge of improving the management of the available resources. Carbon cycle models must take into account the impact of management practices like soil management, fertilizer applications, irrigation, etc. on the productivity and the carbon balance of croplands (Ciais et al., 2010).

Figure 1.1 illustrates the main CO_2 flux components in a cropland carbon budget. Net ecosystem exchange (NEE) is the residual between gross primary production (GPP; gross uptake of CO_2 due to photosynthesis within a given area over a specified timeframe) and ecosystem respiration (R_{eco}- total CO_2 returned to the atmosphere). While GPP originates in photosynthetic organs - mainly in leaves - R_{eco} comes from a variety of plant and microbial processes that are dominated by disparate factors (Falge et al., 2002), and is composed of heterotrophic respiration (R_h- associated with microbial activity) and autotrophic respiration (R_a- associated with plant metabolism for

maintenance and tissue construction). Spatially, R_{eco} is divided into aboveground (leaf and non-leaf shoot respiration) and belowground respiration (total soil CO_2 efflux). R_h is strongly regulated by soil temperature and soil water content while R_a varies as the relative roles of growth and maintenance respiration change (Falge et al., 2002).

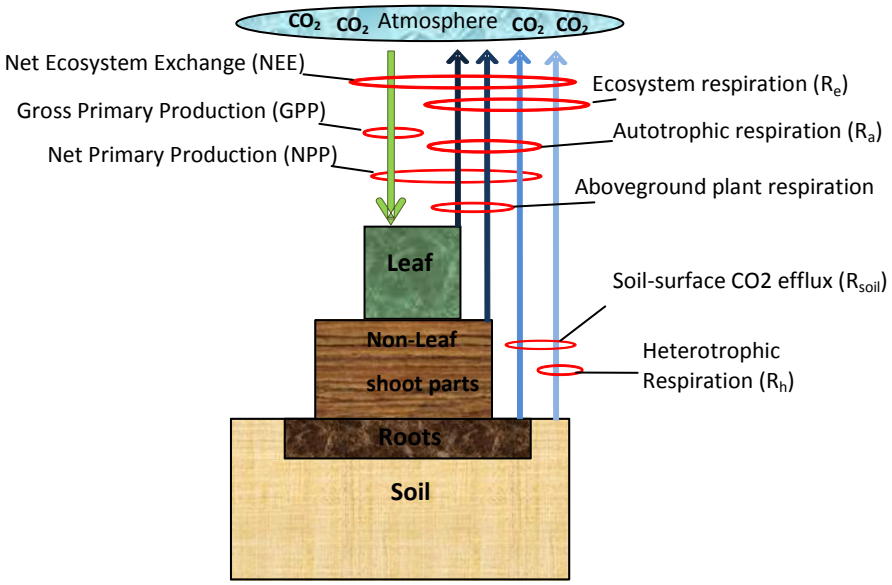

Figure 1.1. Diagram of major components of the carbon balance at ecosystem level. The composite fluxes are defined as; Net ecosystem exchange (NEE), gross primary production (GPP), net primary production (NPP), ecosystem respiration (Reco), autotrophic respiration (Ra) and heterotrophic respiration (Rh).

Whether one measures the rate of photosynthesis occurs or the growth rate of plants, one is concerned with primary production. The quantity of photosynthates not used to generate metabolic energy and therefore available for other processes is called net primary production (NPP). Most of NPP is allocated to the production of biomass (foliage, seeds, fruits, wood, shoots and roots) and hence it is a key part of productivity models. However, a small fraction of NPP is lost via emission of volatile organic C

compounds (which is more important in forestry than cropland), exudation from roots or carbon transfer to root symbionts. These fluxes are very difficult to assess and hence accurate direct measurements of total NPP are impossible in the field (Ciais et al., 2010; Smith et al., 2010). Additionally, some other non-CO_2 losses such as root turnover, harvest, litterfall, etc. occur at different developmental periods of the plant. Due to incomplete accounting of these components there is a systematic underestimation of all reported estimates (Ciais et al., 2010). In croplands, NPP is estimated as follows (Smith et al., 2010):

$$NPP = NPP_{foliage} + NPP_{seed\,/\,fruits} + NPP_{wood\,/\,shoot} + NPP_{roots} \quad (1)$$

Models must partition NPP into its components and relate them with biotic and abiotic drivers to assess the carbon budget of biomes and to explain its spatial and temporal variations, (Law *et al.*, 2002).

Many studies suggest that respiration is a key process in explaining variations in ecosystem productivity and may play a major role in the C balance of ecosystems (Ryan *et al.*, 1997; Cannell & Thornley, 2000; Thornley & Cannell, 2000; Valentini *et al.*, 2000). Despite the importance of R_{eco} in determining the carbon sink/source strength of ecosystems, the control of respiratory CO_2 release by plants is too complicated to be represented mechanistically in current models of annual primary production (Gifford, 2003). R_{eco} integrates many complex biological processes and simple empirical relationships between CO_2 fluxes, and environmental variables don't scale well across space and time (Trumbore, 2006). Flux measurement alone cannot distinguish CO_2 produced by autotrophic sources from the heterotrophic ones (Trumbore, 2006). Soil respiration remains a "black box" because of the shortfalls of existing approaches in partitioning root respiration from total soil-surface CO_2 efflux (Hanson *et al.*, 2000). Therefore, further development and standardization of the current methods is needed for determining NPP.

An alternative adopted by some simple models is the implicit treatment of respiration and photosynthesis by embedding them into the parameters of empirical growth functions. For such purpose the concept of radiation use efficiency has been widely used (RUE; the ratio of biomass accumulation and intercepted radiation). Such models calculate new biomass as the amount of solar radiation intercepted by leaves multiplied by the efficiency of conversion of solar energy into plant dry matter (Monteith, 1972), thus assuming a fixed relationship between photosynthesis and respiration. However, respiration and photosynthesis respond differently to environment variables and hence changes in climate could modify growth rate by altering the balance between both components (Ryan et al., 1997). Therefore, the complex effects produced by the environment on photosynthesis and respiration have to be studied with more sophisticated tools (Gifford, 2003).

The great number of results on leaf photosynthesis contrast with the lack of information available on the photosynthetic performance at the canopy level. Leaf measurements are inadequate when the objective is to quantify fluxes of carbon at stand or tree level (McMurtrie et al., 1992). Scaling up from leaf to canopy by using multi-layer models or models using categories of leaves is always a tricky approach, because of the complexity in the distribution of model parameters within the canopy. The rate of CO_2 fixation is related to the amount of intercepted photosynthetically active radiation (PAR); therefore an accurate canopy radiative transfer model to calculate the light interception at each level in the canopy is essential to estimate canopy photosynthesis (Baldocchi & Hutchison, 1986).

In an irrigated olive orchard, Testi et al. (2008) measured NEE by the eddy covariance technique and soil respiration using a small chamber. At leaf scale, diurnal and seasonal variations of photosynthesis rates have been measured using cuvettes in olive leaves (Angelopoulos, 1996; Moriana *et al.*, 2002; Diaz-Espejo *et al.*, 2006). Lately, a model of leaf photosynthesis was proposed by Díaz-Espejo et al. (2006).

Mariscal et al. (2000) developed a model of PAR interception, and distribution within the canopies of olive orchards. However, although this model could potentially be used to estimate NPP by using RUE approaches (Villalobos et al., 2006), respiration in olive trees has only been studied at leaf level by Diaz-Espejo et al. (2006) and direct measurements at the plant scale have not been presented yet. It is also desirable an improvement in the simulation of the impact that orchard specific characteristics (ground cover, tree size, phenology, soil management, irrigation, etc) have on the carbon balance.

Evapotranspiration and Water Use Efficiency

The availability of water is the factor that restricts most terrestrial plant production on a global scale. Water stress affects many morpho-physiological processes in plants (Hsiao, 1973), reducing vegetative growth and stomatal conductance, and thus, indirectly, photosynthesis and transpiration. Evaluating the impact of water shortage on the carbon and water balance is especially important in the Mediterranean climate, characterized by prolonged dry periods and a large spatial and temporal variability in rainfall amounts. As most of the olive cultivated area is rainfed, a correct assessment of the effect of water stress is essential to quantify gas exchange processes between olive groves and the atmosphere, and to evaluate their productivity, water use and role as a potential sink of carbon.

In absence of water stress, some approaches to the calculation of crop evapotranspiration (ET) have been proposed. The FAO (Food and Agriculture Organization) method uses an empirical crop coefficient (K_c) that depends on canopy cover, crop characteristic, rainfall pattern and irrigation scheduling (the former two factors acting on transpiration and the latter two on the evaporation from the soil) (Villalobos et al., 2000; Orgaz et al., 2007). More advanced methods are biophysical models that calculate transpiration and soil evaporation separately. These models use a

quantitative treatment of transpiration (and CO_2 assimilation) in terms of resistances to gas transport. The resistance of the stomata to the diffusion of CO_2 and water vapour (or its reciprocal: the stomatal conductance) plays a basic role in controlling photosynthesis and transpiration rates; consequently, these models require a parameterization of canopy conductance (G_c) as a function of environmental variables. A specific model of daily G_c for well watered olive canopies formulated by Orgaz et al. (2007) is useful for obtaining good daily E_p predictions, but its applicability to water stress conditions remains an open question.

As stomata are the shared pathway by carbon and water molecules, the resistances to carbon and water fluxes are linked and the relative flux is determined by the gradient of concentrations. While the atmospheric concentration of carbon in air is relatively stable everywhere, the concentration of water vapour in air is widely changing in time and space. All other things being equal, water use efficiency (WUE-the ratio of photosynthesis and transpiration) decreases when the atmospheric water demand increases. Thus, the biomass produced under well-watered conditions can be estimated with a simple function of the relatively stable WUE and the easy-to-obtain vapour pressure deficit (VPD) (Tanner and Sinclair, 1983). If WUE is unresponsive to water stress, or if its response can be predicted, WUE models could still be valid or easily adaptable to water stress conditions; unfortunately this assumptions are not yet confirmed.

Conductance models

The first models aimed to predict stomatal conductance were those using empirical relations with environmental variables. Jarvis (1976) expressed stomatal conductance as a maximum conductance under optimal conditions multiplied by a series of independent stress functions, combined in a multiplicative way (each function representing the

influence of one factor) interacting without feedbacks. The factors were incoming solar radiation, air temperature, vapour pressure deficit and leaf water potential.

It is recognized that stomatal control allows plants to maintain a dynamic balance between carbon gain and water loss. Ball et al. (1987) suggested a semi-empirical model which incorporated the correlation between stomatal conductance (G_s) and photosynthesis (A):

$$G_s = G_0 + a_1 A \frac{RH_s}{C_s} \quad (2)$$

Where G_0 is a residual stomatal conductance (as $A \rightarrow 0$ when incident radiation$\rightarrow$0), a_1 is an empirical coefficient, and C_s and RH_s are the CO_2 concentration and relative humidity (%) at the leaf surface. Subsequent works suggested that stomatal responded to VPD rather than surface relative humidity (Leuning, 1995) :

$$G_s = G_0 + b \frac{A}{(1+\frac{D}{D_0})(C_s - \Gamma)} \quad (3)$$

Where Γ is the CO_2 compensation point, D is the vapour pressure deficit and b and D_o are empirical coefficients.

While the Ball-Berry-Leuning model (BBL) (Eq. 3) successfully describes stomatal response to variations in A, VPD and C_s, its main limitation is that it is not able to predict stomatal closure under soil drying. Therefore, this model is less successful for water-stressed plants where G_s may be relatively insensitive to VPD. Dewar (1995) interpreted the BBL model in terms of a extended guard cell function which was consistent with evidence that stomata respond to transpiration rate (Mott & Parkhurst, 1991) and CO_2 concentrations in the sub-stomatal internal cavity Dewar's model provides an additional reason for the transpiration decline at high VPD, reflecting variations in leaf hydraulic conductance (K). The parameter D_0 of the BBL model was identified by Dewar (1995) as an empirical constant directly related to K.

Olive shows a strong stomatal control on transpiration , thus it is important to assess the stomatal response to environmental variables. Moriana et al. (2002) calibrated and validated three leaf conductance models with field experimental data. Two of them were based on the Leuning model and the third was derived from the Jarvis model. The best performance in the validation tests was found with those obtained from the Leuning model. Testi et al. (2006) found that stomatal response also modulated by endogenous factor in addition to the physiological and environmental variables considered.

Tools for measuring CO_2 and water vapour gas exchange

At the orchard scale, the measurement of CO_2 and water vapour gas exchange can be performed by micrometeorological techniques. The eddy covariance technique for measuring CO_2 exchange, which has been commonly used in recent years (e.g. Euroflux, Ameriflux projects, Falge et al. 2002), gives good measurements of Net Ecosystem Exchange (NEE) but relies on strong assumptions for flux corrections, is difficult to apply and requires large, uniform and flat plots. Leaf cuvette gas analysers have been widely used to measure leaf gas exchange in mature trees (e.g. Moriana et al., 2002); scaling up such observations to canopy level, however, remains a challenge.

A suitable method for measuring the gas exchange of individual trees may be the use of large canopy chambers. Chambers consist of rigid frames covered with plastic films or glass, where the air within the chamber is continuously mixed but isolated from the outside environment. Denmead (1984) classified gas exchange canopy chambers into open (steady-state) and closed (transient-state) systems.

The most critical aspect of almost all chambers is related to the modification of the microclimate during the measurement period. Open systems continuously renew the air inside the chamber while the air flux and gas concentrations in the incoming going and out airstreams are continually measured. This type of chamber is always closed and under constant forced ventilation, thus the radiative and aerodynamic microclimate

around the tree can be very different from the natural undisturbed conditions. In most open chamber systems with air supply, there is a constant overpressure within the chamber to keep the structure inflated. A large air-pumping system with high electrical power is required (Greenwood *et al.*, 1981; Denmead, 1984). Another critical aspect is the radiation environment in the chamber. The walls alter the radiation amount and quality inside the chamber, by reducing direct beam solar radiation and increasing the diffuse component (Leuning & Foster, 1990). Although this problem is common to all chamber types, it is more important in open chambers due to the permanent placement of the enclosure during the measurement period. Enclosing a canopy into a chamber always generate important microclimatic differences between the chamber and the environment (Denmead et al., 1993), thus the enclosing time should be as short as possible.

In this sense, transient-state chambers are placed for a short period of time, while the change in gas concentration is measured. They may be portable and can be placed on the plant for the measurement; they are then removed before the plant can respond physiologically to the changing environment. However, the requirement of being quickly put in place and removed has limited their dimension. Researchers mostly used small closed chambers on plants or pots. Most transitory-closed chambers not exceeding $9m^3$ in volume have been used mainly on herbaceous crops. Enclosing trees with a canopy size larger than $20m^3$ has not yet been tried in a closed system design. A possibility is to use a frame structure, relatively easy to move, which can be opened and closed through revolving top and windows. Such a chamber should ideally disturb minimally the tree environment while open.

Objectives and outline of the thesis

The little information available on the C fluxes of olives at tree scale and its relation with water use encouraged this study.

The main objectives of this thesis were:

(1) To design and test a canopy chamber of the transitory-closed type, large enough to enclose medium-size trees, for measuring with accuracy the carbon and water exchange in the field.
(2) To characterize the carbon exchange of single olive trees and soil under different environmental conditions and its relationship with water use.

The thesis is presented as chapters, each one with specific objectives, which have been structured as peer-reviewed publications. Chapter 1 has already been published and can be found in *Environmental and Experimental Botany 68(2): 131-138*, and Chapters 2 and 3 are being reviewed for publication.

Chapter 1 covers specifically the objective 1 dealing with methodological aspects of the canopy chamber. It describes a series of tests for evaluating physical and physiological responses of the tree to potential environmental modifications of the chamber. The objective 2 is covered across Chapter 2 and 3. In Chapter 2, the carbon assimilation, transpiration and water use efficiency of mature olive trees are investigated in an olive orchard with different irrigation treatments: control with no water stress (CI) and regulated deficit irrigation (RDI). Additionally, a conductance model is proposed to gain insight about the physiological processes leading to variations of the water use efficiency of trees. Chapter 3 focus on how plant and soil respiration patterns regulate the capacity of olive orchards for carbon sequestration and how respiration of olive trees

responds to temperature, phenology and organ plant composition. Finally, a general discussion compiling the main findings is included.

References

Angelopoulos K, Dichio B, Xiloyannis C. 1996. Inhibition of photosynthesis in olive trees (Olea europaea L.) during water stress and rewatering. Journal of Experimental Botany, 47(301): 1093-1100.

Baldocchi DD, Hutchison BA. 1986. On estimating canopy photosynthesis and stomatal conductance in a deciduous forest with clumped foliage. Tree Physiology, 2(1-2-3): 155-168.

Ball JT., Woodrow IE., Berry JA. 1987. A model predicting stomatal conductance and its contribution to the control of photosynthesis under different environmental conditions. . In: Biggins, J. (Ed.), Progress in Photosynthesis Research, vol. 4. : Proceedings of the 7th International Congress on Photosynthesis. Martins Nijhoff, Dordrecht, The Netherlands, pp 221–224.

Cannell MGR, Thornley JHM. 2000. Modelling the Components of Plant Respiration: Some Guiding Principles. Annals of Botany, 85(1): 45-54.

Ciais P, Wattenbach M, Vuichard N, Smith P, Piao SL, Don A, Luyssaert S, Janssens IA, Bondeau A, Dechow R, Leip A, Smith PC, Beer C, Van der werf GR, Gervois S, Van oost K, Tomelleri E, Freibauer A, Schulze ED. 2010. The European carbon balance. Part 2: Croplands. Global Change Biology, 16(5): 1409-1428.

Chalmers DM, Van Heek L. 1981. Control of Peach Tree Growth and Productivity by Regulated Water Supply, Tree Density, and Summer Pruning. Journal of the American Society for Horticultural Science, 106(3): 307-312.

Denmead OT. 1984. Plant physiological methods for studying evapotranspiration: Problems of telling the forest from the trees. Agricultural Water Management, 8(1-3): 167-189.

Denmead OT, Dunin FX, Wong SC, Greenwood EAN. 1993. Measuring water use efficiency of Eucalypt trees with chambers and micrometeorological techniques. Journal of Hydrology, 150(2-4): 649-664.

Dewar RC. 1995. Interpretation of an empirical model for stomatal conductance in terms of guard cell function. Plant, Cell and Environment, 18: 365-372.

Diaz-Espejo A, Walcroft AS, Fernandez JE, Hafidi B, Palomo MJ, Giron IF. 2006. Modeling photosynthesis in olive leaves under drought conditions. Tree Physiology, 26(11): 1445-1456.

Falge E, Baldocchi D, Tenhunen J, Aubinet M, Bakwin P, Berbigier P, Bernhofer C, Burba G, Clement R, Davis KJ, Elbers JA, Goldstein AH, Grelle A, Granier A, Gumundsson J, Hollinger D, Kowalski AS, Katul G, Law BE, Malhi Y, Meyers T, Monson RK, Munger JW, Oechel W, Paw U KT, Pilegaard K, Rannik U, Rebmann C, Suyker A, Valentini R, Wilson K, Wofsy S. 2002. Seasonality of ecosystem respiration and gross primary production as derived from FLUXNET measurements. Agricultural and Forest Meteorology, 113(1-4): 53-74.

Gifford RM. 2003. Plant respiration in productivity models: Conceptualisation, representation and issues for global terrestrial carbon-cycle research. Functional Plant Biology, 30(2): 171-186.

Girona J, Mata M, Fereres E, Goldhamer DA, Cohen M. 2002. Evapotranspiration and soil water dynamics of peach trees under water deficits. Agricultural Water Management, 54(2): 107-122.

Greenwood EAN, Beresford JD, Bartle JR. 1981. Evaporation from vegetation in landscapes developing secondary salinity using the ventilated-chamber technique. III. Evaporation from a Pinus radiata tree and the surrounding pasture in an agroforestry plantation. Journal of Hydrology, 50(C): 155-166.

Hanson PJ, Edwards NT, Garten CT, Andrews JA. 2000. Separating root and soil microbial contributions to soil respiration: A review of methods and observations. Biogeochemistry, 48(1): 115-146.

Hsiao TC. 1973. Plant Responses to Water Stress. Annual Review of Plant Physiology, 24(1): 519-570.

Iniesta F, Testi L, Orgaz F, Villalobos FJ. 2009. The effects of regulated and continuous deficit irrigation on the water use, growth and yield of olive trees. European Journal of Agronomy, 30(4): 258-265.

IPCC, 2007**.** 2006 IPCC Guidelines for National Greenhouse Gas Inventories. Institute for Global Environmental Strategies, Japan.

Jarvis PG. 1976. The interpretation of the variations in leaf water potential and stomatal conductance found in canopies in the field. Phil Trans R Soc Lond B, 273: 593–610.

Law BE, Falge E, Gu L, Baldocchi DD, Bakwin P, Berbigier P, Davis K, Dolman AJ, Falk M, Fuentes JD, Goldstein A, Granier A, Grelle A, Hollinger D, Janssens IA, Jarvis P, Jensen NO, Katul G, Mahli Y, Matteucci G, Meyers T, Monson R, Munger W, Oechel W, Olson R, Pilegaard K, Paw U KT, Thorgeirsson H, Valentini R, Verma S, Vesala T, Wilson K, Wofsy S. 2002. Environmental controls over carbon dioxide and water vapor exchange of terrestrial vegetation. Agricultural and Forest Meteorology, 113(1-4): 97-120.

Leuning R. 1990. Modelling Stomatal Behaviour and and Photosynthesis of Eucalyptus grandis. Australian Journal of Plant Physiology, 17: 159–175. .

Leuning R. 1995. A critical appraisal of a combined stomatal-photosynthesis model for C3 plants. Plant, Cell and Environment, 18(4): 339-355.

Leuning R, Foster IJ. 1990. Estimation of transpiration by single trees: comparison of a ventilated chamber, leaf energy budgets and a combination equation. Agricultural and Forest Meteorology, 51(1): 63-86.

Mariscal MJ, Orgaz F, Villalobos FJ. 2000. Modelling and measurement of radiation interception by olive canopies. Agricultural and Forest Meteorology, 100(2-3): 183-197.

McMurtrie RE, Leuning R, Thompson WA, Wheeler AM. 1992. A model of canopy photosynthesis and water use incorporating a mechanistic formulation of leaf CO2 exchange. Forest Ecology and Management, 52(1-4): 261-278.

Monteith JL. 1972. Solar Radiation and Productivity in Tropical Ecosystems. Journal of Applied Ecology, 9(3): 747-766.

Moriana A, Villalobos FJ, Fereres E. 2002. Stomatal and photosynthetic responses of olive (Olea europaea L.) leaves to water deficits. Plant Cell and Environment, 25(3): 395-405.

Moriana A, Orgaz F, Pastor M, Fereres E. 2003. Yield responses of a mature olive orchard to water deficits. Journal of the American Society for Horticultural Science, 128(3): 425-431.

Mott KA. 1988. Do Stomata Respond to CO2 Concentrations Other than Intercellular? Plant Physiology. 86(1): 200-203.

Mott KA, Parkhurst DF. 1991. Stomatal responses to humidity in air and helox. Plant, Cell and Environment, 14(5): 509-515.

Orgaz F, Villalobos FJ, Testi L., Fereres E. 2007. A model of daily mean canopy conductance for calculating transpiration of olive canopies. Functional Plant Biology, 34: 178-188.

Ryan MG, Lavigne MB, Gower ST. 1997. Annual carbon cost of autotrophic respiration in boreal forest ecosystems in relation to species and climate. Journal of Geophysical Research D: Atmospheres, 102(24): 28871-28883.

Smith P, Lanigan G, Kutsch WL, Buchmann N, Eugster W, Aubinet M, Ceschia E, Béziat P, Yeluripati JB, Osborne B, Moors EJ, Brut A, Wattenbach M, Saunders M, Jones M. 2010. Measurements necessary for assessing the net ecosystem carbon budget of croplands. Agriculture, Ecosystems and Environment, doi: DOI: 10.1016/j.agee.2010.04.004.

Tanner CB, Sinclair TR. 1983. Efficient water use in crop production: research or re-search? In: Taylor HM, Jordan WR, Sinclair TR (Eds), Limitations to Efficient Water Use in Crop Production. ASA, CSSA, SSSA, Madison, Wisconsin, USA, pp. 1-27.

Testi L, Orgaz F, Villalobos FJ. 2008. Carbon exchange and water use efficiency of a growing, irrigated olive orchard. Environmental and Experimental Botany, 63(1-3): 168-177.

Testi L, Orgaz F, Villalobos FJ. 2006. Variations in bulk canopy conductance of an irrigated olive (Olea europaea L.) orchard. Environmental and Experimental Botany, 55(1-2): 15-28.

Thornley JHM, Cannell MGR. 2000. Modelling the components of plant respiration: Representation and realism. Annals of Botany, 85(1): 55-67.

Trumbore S. 2006. Carbon respired by terrestrial ecosystems - Recent progress and challenges. Global Change Biology, 12(2): 141-153.

Valentini R, Matteucci G, Dolman AJ, Schulze ED, Rebmann C, Moors EJ, Granier A, Gross P, Jensen NO, Pilegaard K, Lindroth A, Grelle A, Bernhofer C, Grünwald T, Aubinet M, Ceulemans R, Kowalski AS, Vesala T, Rannik Ü, Berbigier P, Loustau D, Guomundsson J, Thorgeirsson H, Ibrom A, Morgenstern K, Clement R, Moncrieff J, Montagnani L, Minerbi S, Jarvis PG. 2000. Respiration as the main determinant of carbon balance in European forests. Nature, 404(6780): 861-865.

Villalobos FJ, Orgaz F, Testi L, Fereres E. 2000. Measurement and modeling of evapotranspiration of olive (Olea europaea L.) orchards. European Journal of Agronomy, 13(2-3): 155-163.

Villalobos FJ, Testi L, Hidalgo J, Pastor M, Orgaz F. 2006. Modelling potential growth and yield of olive (Olea europaea L.) canopies. European Journal of Agronomy, 24(4): 296-303.

Chapter 1: A large Closed Canopy-Chamber for measuring CO_2 and Water Vapour exchange of Whole trees

Abstract

A transient-state chamber was developed to measure canopy gas exchange of single trees in the field. The chamber, with a volume of 41.6 m^3, is designed to enclose a medium-size orchard tree; chamber top and windows can be left open, causing minimum disturbance to the tree environment. Transitory closures allow simultaneous measurement of CO_2 exchange and transpiration of the enclosed tree. The chamber was tested during a 2-year study in an olive orchard submitted to different irrigation treatments: control with no water stress (CI) and regulated deficit irrigation (RDI). Leakage had a minimal impact on flux calculations (0.8% min^{-1}); adsorption was not detectable. Maximum increases in canopy temperature of 0.58 ^oC min^{-1} for CI and 1.3 ^oC min^{-1} for RDI generated very small effects on fluxes. Changes in the transpiration rate induced by the chamber's modification of the canopy environment were evaluated by continuous sap flow measurements with heat pulse gauges inserted in the trunk of two trees enclosed by chambers. Results showed a sap flow decrease of about 8% after 180 s of chamber closure. The artificial turbulence generated by fans into the chamber to facilitate air mixing did not alter the transpiration rate. The enclosure had a very small impact on the tree canopy conductance (G_c). The initial lag and mixing time was estimated as 30 s; the optimal duration of the calculation window was 70 s. Hourly carbon assimilation (A), transpiration (E), and water use efficiency (WUE) for two olive trees in the field subjected to different levels of water stress were measured.

1. Introduction

The measurement of carbon assimilation and water use of trees is critical to understand the processes leading to their carbon and water balance, but is difficult to perform at the whole-plant level. At leaf level, cuvette gas analysers have been used to measure leaf gas exchange of mature trees (e.g. Moriana et al., 2002). Scaling up such observations to canopy level, however, remains a challenge. A suitable method for measuring the gas exchange of individual trees is the use of large canopy chambers. Nevertheless, there are practical difficulties in the design, construction and operation of chambers large enough to enclose a whole tree.

Denmead (1984) classified gas exchange canopy chambers into open (steady-state) and closed (transient-state) systems. The open system continuously renews the air inside the chamber while measuring the gas concentration in the entry and exit airstreams. This type of chamber is always closed and under constant forced ventilation, thus the radiative and aerodynamic microclimate around the tree can be very different from the natural undisturbed conditions. In most open chamber systems with air supply, there is a constant overpressure within the chamber to keep the structure inflated. Furthermore, steady airflow is often higher than natural wind speed and a suitable ventilation rate is difficult to get. A large air-pumping system with high electrical power is required (Greenwood et al., 1981; Denmead, 1984). Another critical aspect is the radiation environment in the chamber. The walls alter the radiation amount and quality inside the chamber, by reducing direct beam solar radiation and increasing the diffuse component (Leuning and Foster, 1990). Although this problem is common to all chamber types, it has a bigger effect in open chambers due to the permanent placement of the enclosure during the measurement period. Enclosing a canopy into a chamber always generate important microclimatic differences between the chamber and the environment (Denmead et al., 1993), thus the enclosing time should be as short as possible.

Transient-state closed chambers are closed for a short period of time (minutes), while the change in gas concentration is measured. They are portable and can be placed on the plant for the measurement; they are then removed before the plant can respond physiologically to the changing environment. Reicosky and Peter (1977) described this type of easily moving chambers for measuring transpiration on field plots. More recently, this type of canopy-chamber has been automated for continuous measurements of CO_2 and H_2O fluxes (Steduto et al., 2002). The simplest method for calculating gas fluxes with a closed chamber is by performing a linear regression of gas concentration with time. The principles involved in this approach have been reviewed by Jarvis et al. (1971). Later, different methods of calculation have been proposed by Reicosky et al. (1990) and Wagner et al. (1997). Wagner and Reicosky (1992) studied the chamber effect on the plant environment, measuring changes in canopy photosynthesis rates and evapotranspiration rates. The relevant factors involved are mostly CO_2 and water vapour concentration, canopy temperature, air temperature, canopy boundary layer conductance, stomatal conductance (g_l) and vapour pressure deficit (VPD).

Researchers mostly used small closed chambers on plants or pots (Reicosky and Peters, 1977; Reicosky, 1990a, 1990b; Held et al., 1990; Pickering et al., 1993; Grau, 1995; Steduto et al., 2002; McLeod et al., 2004). Most transitory closed chambers not exceeding 9 m^3 in volume are reported in the literature, used mainly on herbaceous crops. Most of them have been evaluated comparing with accurate and direct methods such as a weighing lysimeter (Reicosky et al., 1983; Pickering et al., 1993) and heat pulse sap flow (Dragoni et al., 2005) or micrometeorological methods (Held et al., 1990; Dugas et al., 1991; Steduto et al., 2002; McLeod et al., 2004). Enclosing trees with a canopy size larger than 20 m^3 has not yet been tried in a closed system design. A possibility is to use a frame structure, relatively easy to move, which can be opened and closed through revolving top and windows. Such a chamber should ideally disturb minimally the tree environment while open.

The objectives of this work were: a) to design a chamber of the transitory-closed type, large enough to enclose adult orchard trees; b) to test the chamber for measuring canopy gas exchange in the field, and c) to evaluate physical and physiological responses of the tree to potential environmental modifications of the chamber.

2. Material and Methods

2.1. Chamber description

The transient-state closed chamber presented here is a variation of the chamber described by Reicosky and Peters (1977), compatible for simultaneous measurement of both CO_2 and water vapour exchange. The chamber consists of a hexagonal prism with base of 10.4 m^2 and height of 4 m; the volume - 41.6 m^3 - is enough to enclose a mature tree of most of the cultivated fruit species (Fig. 1). The walls are assembled with 12 sections of rigid aluminium frames (2 m x 2 m) that are easy fitted and screwed together. The whole chamber is mounted around the tree on a firm stainless steel frame with sharp supports sunk in the soil. The bottom is sealed around the tree trunk through a thick polyethylene panel; thus, fluxes from the soil are excluded. The top is made by two trapezium-shaped windows. The top and four of the six walls are hinged windows, which allow quick opening and closing operation. Windows and tops are made of Llumar® "NRS90 clear" polyester film of 75 μm thickness, stretched and fixed to the aluminium frames; this film was chosen for its excellent broadband transmittance. The top and upper windows can be quickly moved manually by extended handles. The chamber contains four 80 W fans of 40 cm diameter attached to the basal frame, mixing the air inside the chamber during the closed period (which in normal operating conditions does not need to exceed three minutes). A vacuum pump circulates the air through the sampling circuit: the air is taken from inside the chamber through many intake points, spatially distributed through the chamber volume (Fig. 1.2) and is then returned to the chamber. A sample of 1 L min^{-1} of this air flow is sniffed using a small

pump and diverted to a CO_2/H_2O infrared gas analyser (IRGA) (model LI-COR LI-840, Lincoln, NE, USA) which measures CO_2 and water vapour concentrations simultaneously at 1 Hz sampling rate; the output is recorded by a datalogger (model CR23X, Campbell Scientific, Logan, UT, USA). The IRGA, datalogger and small pump were powered by 12V batteries. The vacuum pump and the fans are powered using a small portable A/C generator.

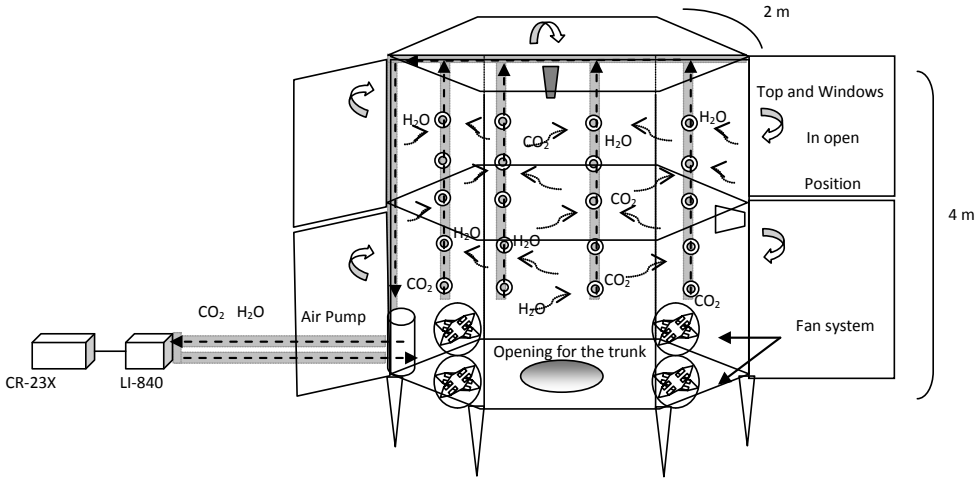

Figure 1.2. Schematic drawing of the chamber, showing the opening windows and other components. The arrows indicate the air flow through the sampling circuit, and its return to the chamber.

An infrared thermometer (model IRTS-P, Apogee, UT, USA) is mounted on the centre of the chamber top, facing downwards, to measure the temperature of the canopy. The sensor has a 52º field of view, and can be aimed and adjusted in height for optimal foliage targeting. The air temperature and relative humidity were measured inside the chamber with a combined probe (model CS215, Campbell Scientific, Logan, UT, USA) placed near the top of the chamber into a radiation shield. Air temperature and relative humidity were recorded every 4 s in order to evaluate changes in vapour pressure deficit (VPD) in the confined atmosphere. Two complete chambers were built and arranged for simultaneous measurements in two trees.

The chamber is normally kept with all the windows and top in the open position. Just before measuring, the windows and top are closed and fans are turned on. The CO_2 and water vapour concentrations, measured by the IRGA, change steadily after a short lag time. The rate of change of both gases with time is the apparent flux of assimilation and transpiration, respectively, of the tree. The gas fluxes are transformed to mass basis per surface and time unit, simultaneously, following Jarvis et al. (1971), and include the correction for air temperature and atmospheric pressure inside the chamber (Reicosky et al., 1990). The method and calculation window used for assessing CO_2 exchange and transpiration rates are discussed later. After the measurement is completed, the chamber goes back to the open position, with the fans off until the following measurement.

2.2. Chamber tests

A series of experiments were carried out in the laboratories of the Instituto de Agricultura Sostenible (IAS) of Cordoba, Spain, to assess the potential measurement errors. Three tests were aimed to quantify some potential disturbances that the closed chamber could cast over the internal environment, namely: a) gas leakage; b) gas adsorption by the chamber walls and elements and c) alteration of internal radiation levels by walls and frames. In addition, two field tests were designed to assess the magnitude of some unwanted effects that the confined environment could carry over the physiology of the enclosed canopy, in particular: a) effect over the foliage temperature and b) effect over the transpiration rate and the canopy conductance. These field test were conducted in an irrigated olive (*Olea europaea* L. 'Arbequino') orchard planted in summer 1997 with a 3.5 m x 7 m spacing, located in Córdoba, Spain (37.85ºN, 4.8ºW, altitude 110 m). Two trees in different water conditions (one from an irrigated control treatment labelled as CI and one -water stressed- from a Regulated Deficit Irrigation treatment labelled as RDI) were chosen to test the chamber effect on physiology; two chambers, sharing the same IRGA, were mounted around these trees, allowing quasi-

simultaneous operations. The Plant Leaf Area of the trees was measured using a Plant Canopy Analyser (model LAI2000, LI-COR, Lincoln, NE, USA).

For all tests, the IRGA was operated in the range of ±1000 µmol mol^{-1} for CO_2 and ±80 mmol mol^{-1} for water vapour. A two-point calibration procedure was carried out in the laboratory before measurements operations. A weather station, located less than 300 m from the experimental plot provided additional meteorological data.

2.2.1. Leakage test

A chamber operating as a closed system has to ensure that the envelope is tightly sealed, as leakages would alter the rate of change of the sampled gases inside the chamber. The leakage test for CO_2 was performed by injecting an amount of CO_2 into the empty chamber. The decline in [CO_2] was then measured for 24 minutes. The leakage error was deduced from the differential equation:

$$\frac{dC_i}{dt} = \frac{-\theta(C_i - C_a) + F}{V} \quad (1)$$

Where θ is the leakage coefficient (m^3 s^{-1}, specific of a given chamber); C_i and C_a, are the CO_2 concentrations inside and outside the chamber, respectively (µmol CO_2 mol^{-1}); V is the chamber volume (m^3); F is the assimilation ($F < 0$) or respiration ($F > 0$) flux (µmol CO_2 mol^{-1} m^3 s^{-1}). Thus, considering $F = 0$ (empty chamber) and integrating time t_1 to t_2 (s), the leakage coefficient (m^3 s^{-1}) is:

$$\theta = \frac{-V}{\Delta t} \ln\left(\frac{C_{i2} - C_a}{C_{i1} - C_a}\right) \quad (2)$$

Combining F and θ from the differential equation:

$$F = -\theta(C_i - C_a) + V\frac{dC_i}{dt} \tag{3}$$

Where the first term on the right of Eq. (3) represents the error and the second term the apparent flux (F'). Hence, for an interval time Δt and if $C_{i1} \approx C_a$, the relative error (ε) will be:

$$\varepsilon = \frac{-\theta(C_i - C_a)}{-\theta(C_i - C_a) + F'} = \frac{\theta\,\Delta t}{2V} \tag{4}$$

The relative error is thus not dependent on the concentration gradient, but only on the duration of the closure period. We assume that under field conditions (with non-zero wind outside and the fans on) the leakage is mainly an airflow rather than a diffusive process, thus the relative error is assumed to be equally valid for water vapour.

2.2.2. H_2O Adsorption and radiation transmissivity tests

The potential error due to H_2O adsorption on the chamber walls and in the sampling tubing was evaluated by injecting a known mass of water vapour generated with a conventional humidifier machine into the closed chamber, then measuring the water vapour concentration after one minute. The test was repeated 12 times, injecting different amounts of water vapour. As the leakage flow was already known from the leakage test, the adsorbed mass was estimated as difference between the measured mass flow and the leakage flow.

Concurrent measurements of solar radiation inside and outside the chamber were performed to determine the attenuation of the solar radiation with the change in sun angle. Eight measurements were taken at different hours on a clear-sky day, using with a red/far red sensor (model SKR-110, Skye Instruments, UK), sensible to the wavelengths of 660 and 730 nm. The average value for the attenuation of the solar

radiation by the Llumar® film resulted in 9.9% and 14.6% in the wavelengths 660 and 730 nm, respectively. The transmissivity of the chamber to the total photosinthetically active radiation (PAR) measured by a quantum meter (model QMSW-SS, Apogee Instrument Inc, Logan UT, USA) resulted 90% in average during a clear-sky day.

2.2.3. Canopy and air temperature increase

Perhaps the main difficulty associated with transient closed chambers is that they are expected to increase quickly the canopy and internal air temperature by reducing convection and decreasing the long-wave radiation loss: consequently the foliage gas exchange may depart from natural conditions. The infrared thermometer installed permanently on the chamber top permitted to monitor closely the canopy temperature, while the temperature and VPD of the stirred air inside the chamber was measured by the combined air temperature and humidity probe. Due to the effect of transpirational cooling, the temperature of the enclosed canopy varies also with the transpiration rate. For this reason, the data were collected quasi-simultaneously in both water-stressed and unstressed trees. Data of air and canopy temperature increase for 175 standard chamber operations in the field (closing, measuring during about three minutes and unclosing) were collected in different environmental conditions, from July to October 2007.

2.2.4. Comparison with heat pulse sap flow

This test was aimed to find out if the enclosure of the canopy into the chamber would have a significant effect on the tree transpiration rate, due to the general alteration of the canopy environment. The sap flow velocity was measured using heat pulse gauges inserted in the trunk of an olive tree where the chamber had previously been mounted. The heat pulse velocity measurements were taken at 3-minute intervals, from 930 h to 1230 h on 5 July 2007, obtaining a quasi-continuous trace of sap velocity; at the same time the chamber was operated (closed) in periods of different duration (from about 252 to 464 s). The sap flow sensors were designed in the IAS laboratory. Each sensor

consisted of three stainless steel needles of 2-mm diameter: a 4.8 W heater and two temperature probes. The temperature probes were inserted into the trunk at a distance of 10 and 5-mm down- and up-stream from the heater, respectively. Each temperature probe contained 4 Type E (chromel-constantan) thermocouple junctions, spaced 10 mm along the needle, which were sampled independently to obtain heat pulse velocities at 4 depths. Further details on the sap flow system may be found in Testi and Villalobos (2009).

2.3. Duration of the calculation window and gradient effect

Before the exchange flux of a gas can be reflected by the rate of change of its concentration in the confined atmosphere, there is always a lag time (the time for the gas to reach the sensing chamber of the IRGA through the sampling tubing) and a stirring time (the time needed for the fans to break the leaves boundary layer and mix the confined atmosphere effectively). The combination of lag and stirring time was evaluated by injecting a pulse of CO_2 inside the empty chamber, and then monitoring the CO_2 concentration until a stable rate of change (only depending on leakage) was reached. This point was considered the beginning of the calculation window (the period of time which measurements are then actually used to calculate the flux).

In principle, transient state chambers induce modifications in the internal environment right after closing. Apart from the possible thermal and aerodynamic effects over the canopy, the variation itself in the concentration of the gases inside the chamber reduces the gradient between the leaves and the sampled atmosphere: the effect is that the process under study (either transpiration or photosynthesis/respiration) is deviated from the undisturbed rate. Even keeping every other factor unchanged, the rate of change of the gas concentration versus time is unlinear, because of gradient variation. Ideally, the undisturbed rate of gas exchange is the initial slope of this unlinear relation.

The concentration regression method (CR) calculates the changes of gas concentration (CO_2 or water vapour) as the slope of the least squares regression line relating gas concentration to time (Meyer et al. 1987), thus ignores the nonlinearity. On the contrary, the quadratic regression model (QR) proposed by Wagner et al. (1997) adds a second-order term (observer effect), and was used to account for the influence of the physical disturbances that result in non linear gas concentration change inside of closed systems. The quadratic model used was:

$$\begin{aligned} \left[CO_2 \text{ or Water Vapor}\right] &= \text{linear process} + \text{observer effect} \\ &= a + bt + ct^2 \end{aligned} \tag{5}$$

The change of CO_2 and water vapour concentration versus time will be:

$$\frac{d\left[CO_2 \text{ or Water Vapour}\right]}{dt} = b + 2ct \tag{6}$$

Where *b* and *c* are the regression coefficients of the quadratic function. Then, for time zero, the slope (which is the exchange rate unaffected by chamber closure) is simply equal to *b*. The quadratic regression deals with the error of gradient reduction, but at the price of ascribing a paramount importance to the determination of the time zero, because only the slope in this point is the sought gas exchange rate.

The optimal duration of the calculation window was obtained empirically, by testing the stability in the rate of change of the sampled gas concentration with time. The test consisted in comparing the CR method with the QR method for 175 field measurements obtained in 2006 and 2007 in olive trees in different water status.

2.4 Canopy Conductance approach.

A simplified approach for calculating canopy conductance (G_c) in a well-ventilated canopy chamber is the inversion of the "imposed evaporation" Penman-Monteith Equation (e.g. Villalobos et al., 2000; Orgaz et al., 2007). Thus, canopy conductance G_c (m s^{-1}) is:

$$G_c = \frac{\gamma}{\rho C_p} \frac{\lambda E}{D} \tag{7}$$

Where, γ is the psychometric constant (kPa K^{-1}), C_p the specific heat capacity of air at constant pressure (kJ kg^{-1} K^{-1}), ρ the air density (kg m^{-3}), D the vapour pressure deficit (kPa), λ is the specific heat of vaporisation (MJ kg^{-1}) and E the transpiration rate (kg m^{-2} s^{-1}).

Changes in G_c may be evaluated continuously during the measurement combining Equations (6) and (7) with the rate of change of water vapour concentration and VPD versus time:

$$G_c = \frac{\gamma}{\rho C_p} \frac{\lambda(b + 2ct)}{D} \tag{8}$$

Where b and c were already defined.

3. Results

3.1. Leakage and adsorption tests

The amount of CO_2 injected into the chamber produced an increase of 90 µmol CO_2 mol^{-1}. The decline in [CO_2] was then measured for 24 minutes with the IRGA (Fig. 2.2).

The concentration trace was divided into intervals of 2 minutes and leakage coefficients were calculated for each interval using Equation 2. The mean leak coefficient calculated for all intervals resulted 0.011 m^3 s^{-1} (Fig. 2.2). The average relative error (ε) due to leakage in the calculation of fluxes (Equation 4) was 0.8 % min^{-1}.

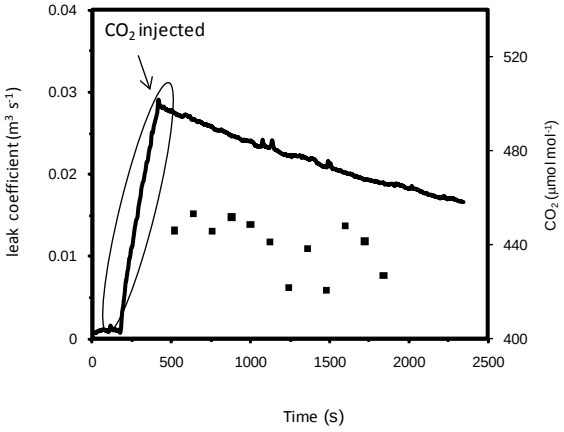

Figure 2.2. Leakage test: the decline in the internal CO_2 concentration after a CO_2 injection (solid line). The squares show the leak coefficients calculated for 2-min intervals. The test yielded a relative error of 0.8% min^{-1}.

The amount of water vapour injected with a humidifier varied from 13.8 g to 91.1 g. Those water vapour injections increased the [H_2O] of the chamber from 0.45 to 3.04 mmol H_2O mol^{-1}. The IRGA measured very accurately the amounts of water vapour added to the chamber (Fig. 3.2); no significant deviation from the 1:1 line possibly due to adsorption was observed, in the explored range.

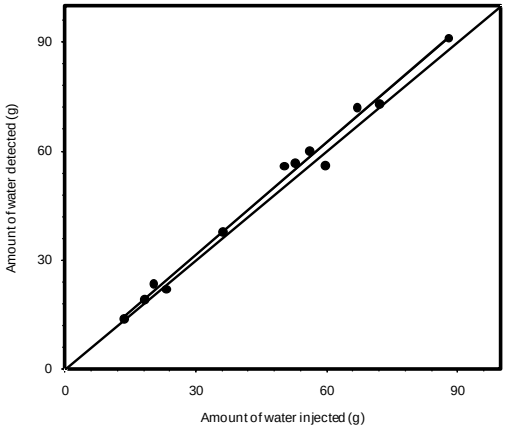

Figure 3.2. Adsorption test and response to a known amount of water vapour, manually injected. The equation of the regression line was: y = 1.0295x + 0.5556 (R^2 = 0.99, N = 12).

3.2. Effect on canopy and air temperature

Figure 4 represents the rate of change, during the closure operation, of the enclosed canopy temperature (Figure 4.2a) and air temperature inside the chamber (Figure 4.2b) versus the free air temperature registered at the weather station nearby. The most significant increases in canopy temperature were appreciated in trees under water stress (RDI treatment) during the hot season. The maximum canopy temperature increases resulted 0.58 ºC min^{-1} in the well-irrigated control (5 July 2007, 900 h) and 1.3 ºC min^{-1} in the RDI (6 September 2007, 1300 h). The higher increments were observed during the hot season with high VPD in the afternoon (Figure 4.2a). On the contrary, the internal air temperature rate of increase seems independent from the tree water status (Figure 4.2b); also, the free air temperature affects weakly the rate of increase of the air inside the chamber. In general, the air inside the closed chamber heats up slowly: the average air temperature increases were 0.22 ºC min^{-1} and 0.14 ºC min^{-1} for CI and RDI, respectively. The maximum values were found close to 0.7 ºC min^{-1}, for both treatments, recorded on 2 August.

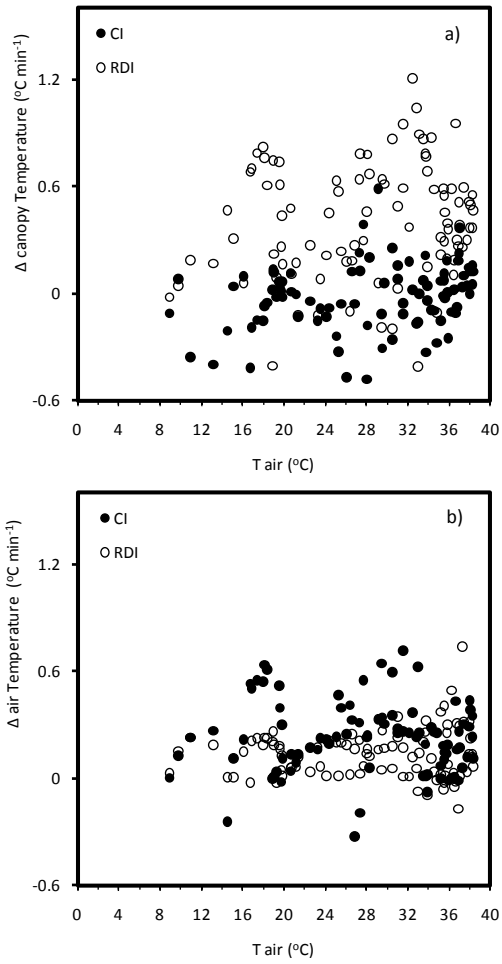

Figure 4.2. Increases in canopy temperature (a) and air temperature (b), expressed in ºC increase per minute since chamber closure, both plotted vs. air temperature outside the chamber. Measurements taken during 2007 in olive. Black circles: well irrigated (CI) treatment; white circles: water stressed (RDI) treatment.

3.3. Effect on transpiration rate

The velocity of the sap through the xylem vessels is proportional to the transpiration flux, if the trunk diameter and capacitance don't change; thus, in the short term, variations in the sap velocity are assumed to be proportional to variations in the transpiration rate. Figure 5.2a shows sap velocity data from 930 h to 1230 h at 30-minute intervals; the vertical strips indicate the periods when the chamber was closed.

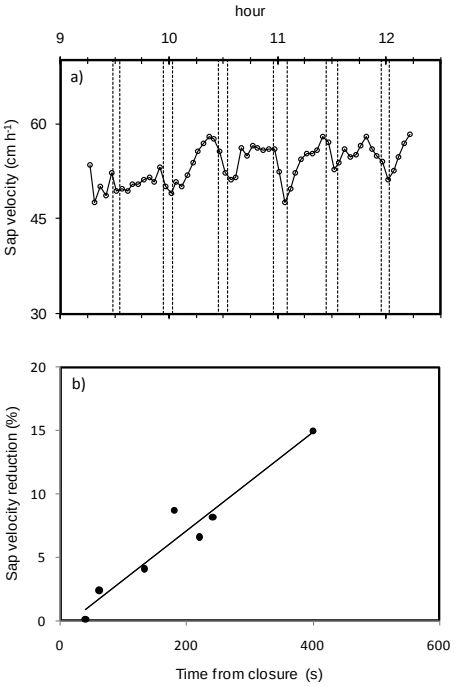

Figure 5.2. (a) Sap velocity measured with compensated heat-pulse sap flow probes in an olive tree during chamber closure and reopening. The vertical dashed lines delimit the closure periods of different durations (from 252 s (at 930 h) to 464 s (at 1100 h). (b) Relation between time from closure and reduction in sap velocity. The equation of the regression line is: $y = 0.0388x - 0.62$ ($R^2 = 0.94$, N=7).

Apparently, the sap flow velocity is slightly reduced during and immediately after the closing. The velocity is then normally recovered few minutes after the chamber re-opening. Figure 5.2b shows the relative decrease in sap flow velocity vs. duration of the closure. The maximum relative decrease in transpiration rate (15%) occurred for the longest closure time (400 s). For a normal measurement period of 180 s the maximum decrease was 8%.

Figure 6.2 details the changes occurring in vapour pressure deficit (VPD), water vapour concentration and canopy conductance for the longest duration of chamber closure shown in Figure 5.2a (400 s, at 1104 h). The canopy conductance (Eq. 8) decreases from 2.1 to 1.7 mm s^{-1} (19%), but mainly in the late part of this long closure period: in the first 200 s of chamber closure G_c is practically constant.

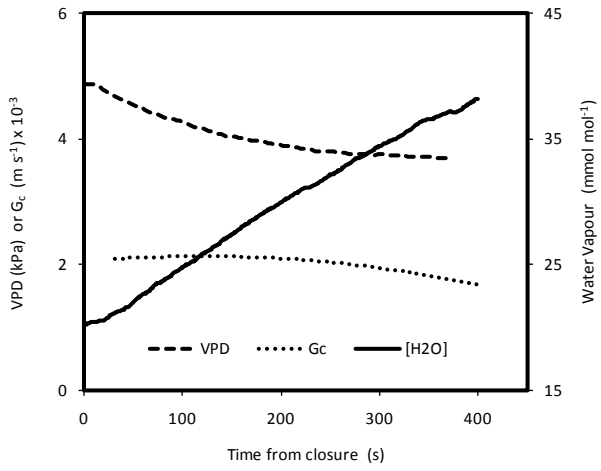

Figure 6.2. Changes recorded in some chamber environment variables during the closure operation at 1100 h shown on Fig. 5.2: vapour pressure deficit (VPD - dashed line), water vapour concentration ([H_2O] - solid line) and canopy conductance (G_c - dotted line).

During closure, a decrease 1.2 kPa in VPD occurs, as a consequence of the increase of water vapour concentration from 20.2 to 38.2 mmol mol^{-1}. Although not noticeably, the plot of water vapour concentration is unlinear, due to the effect of gradient reduction. The error in the calculation of the water vapour flux induced by the use of a linear regression instead than a QR exceeds 20%, over this long measurement.

3.4. Adjusted measurement window and minimisation of the gradient effect

The lag and mixing time measured were always found in the range between 20 and 40 s after closure; the beginning of the calculation window was assumed 30 s in average. The optimal duration of the measurement windows was adjusted to 70 seconds: with that sampling duration, the underestimation of the gas exchange fluxes calculated with the linear regression with respect to the quadratic method on the 175 field measurement was minimal (3.7% for CO_2 and 5.6 % for water vapour), ensuring that the nonlinearity in the gas concentration data with time had the less possible impact on the calculation of fluxes.

3.5. An example of canopy gas exchange measurements

Figure 7.2 shows the daily time course of assimilation, (*A*, Figure 7.2a) transpiration *(E*, Figure 7.2b), and canopy conductance (G_c, Figure 7.2c) for two trees on 6 September 2007 when the difference in water status between the Control Irrigation (CI) and Regulated Deficit Irrigation (RDI) treatments was maximum. The Plant Leaf Area of the trees resulted: 20.19 m^2 for CI and 26.87 m^2 for RDI. The total CO_2 net uptakes during the measurement period (from 630 h to 1730 h solar time) were very different: 21.68 and 9.86 g CO_2 m^{-2} for CI and RDI trees, respectively. The peak assimilation rate occurred in the morning, with 2.56 g CO_2 m^{-2} h^{-1} (CI, at 1100 h) and 1.33 g CO_2 m^{-2} h^{-1} (RDI, at 830 h), then it decreased during the afternoon. Transpiration showed a different diurnal pattern: in the CI it reached its maximum value (0.3 L m^2) at 1300 h, remaining high until late afternoon; on the contrary, in the stressed tree *E* was relatively flat,

reaching the maximum value in the morning at 1045 h. The total water transpired by the CI tree during the day was 2.34 L m^{-2}, whereas the stressed RDI tree only lost 0.77 L m^{-2} of water over the same time.

The diurnal course of canopy conductance in Figure 7.2c was calculated from Equation 7 (where t (s) is taken equal to the beginning of the calculation window), using the same gas exchange measurements shown in Figure 7.2a and b. Right after sunrise, the G_c in the well-irrigated tree increases steeply with global radiation; the water stressed tree showed the same behaviour, but to a much lesser extent. The time of maximum G_c is reached in the RDI treatment first (1.9 mm s^{-1} at 710 h) than in the well irrigated CI (5 mm s^{-1} at 800 h). After reached the peak, G_c followed a light decreasing during the daytime period in both trees.

Measuring CO_2 and water vapour exchange at the same time allows the measurement of the instantaneous Water Use Efficiency (WUE), i.e. the ratio of assimilated CO_2 per unit transpired water.

The water use efficiency of the gas exchange measurements of 6 September 2007 is presented in Figure 8.2, plotted vs. VPD. For both trees WUE show an inverse relation with VPD, with a curvilinear pattern. The highest plotted values of WUE are early-morning values, with low VPD. The WUE was higher in the water stressed tree (RDI) than in the control (CI) for all the duration of the day; the daily values of WUE were 9.25 and 12.74 g CO_2 L^{-1} of transpired water for the CI and RDI trees respectively.

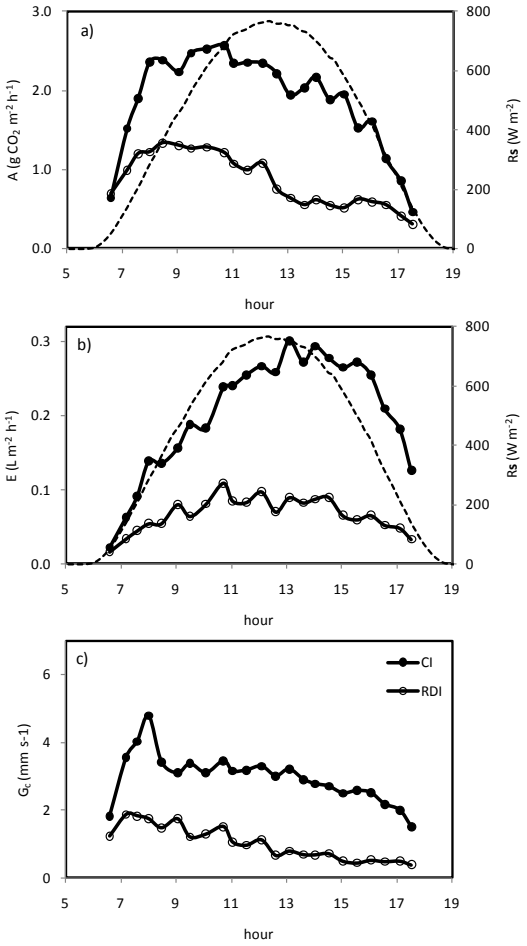

Figure 7.2. Diurnal courses of: (a) canopy net assimilation; (b) transpiration; (c) canopy conductance in olive trees under different water status, all measured with the gas exchange chamber on 6 September 2007. Black circles: well irrigated (CI treatment); white circles: water stressed (RDI treatment). The global radiation is also shown (dashed line). Fluxes were determined using a calculation window of 70 s.

4. Discussion

The large volume of the chamber determines that the relative error associated to CO_2 leakage flux (0.8 % min^{-1}) is very small, in particular considering the short time required to obtaining a measurement. The leakage coefficients (Figure 2) calculated for each period into which the experiment was divided show an evident variability (Figure 2), which is probably a consequence of the pressure waves induced by wind gusts around the chamber walls (the experiment was conducted in open air, under field conditions). The leakage error is lower than those reported in the literature for transient-state closed chambers. Held et al. (1990) calculated a leak flow error of 4% min^{-1} injecting CO_2 into the chamber, from an initial concentration gradient between the chamber and the ambient air of 50 μmol mol^{-1}. Grau (1995), with an open-bottom chamber inserted on a bed of sterilised sand, evaluated an underestimation error due to leakage of 5% in a standard measure with a small (62.5 L) chamber. Steduto et al. (2002) determined a maximum relative error of 4% min^{-1} for both CO_2 and water vapour, under different gradients of gas concentrations.

The linear relationship (Figure 3.2) between the water vapour amounts injected into the chamber and the ones detected by the IRGA indicated that the chamber is capable to measure correctly the changes in water vapour concentration. The slope and intercept of the regression line are not significant, indicating that errors of water vapour absorption on the chambers walls and in the tubing are either absent or too small to be detectable. This result is not surprising, as adsorption is expected to be a function of the surface area per unit volume, thus must be low in large chambers.

The attenuation of the global radiation measured under clear sky conditions (9.9-14.6%) is similar to those found in literature (Reicosky et al., 1983; Held at al., 1990; Pickering et al., 1993; Steduto et al., 2002; Dragoni et al., 2005). Pickering et al. (1993) reported that corrections to transpiration rates appear to be unnecessary for radiation attenuation

of 10%. Nevertheless, according to Held et al. (1990), a simple correction in net CO_2 assimilation may be applied to the calculated fluxes, once it is known the light response curve of the assimilation in the canopy under study. The transmittance of the film in the measured wavelengths exceeded manufacturer's rate (87% for the whole visible spectrum).

The maximum recorded increase in canopy temperature during closure (1.3 ºC min^{-1}, Fig. 4.2) is significantly lower than those (2 to 4 ºC min^{-1}) reported in the literature with closed chambers (McPherson et al., 1983; Reicosky, 1990 a, b; Wagner and Reicosky, 1992; Grau, 1995; Steduto et al., 2002). The large volume of the chamber and the low rate of air temperature increase (maximum value of 0.7 ºC min^{-1}) maintains the effectiveness of the convective cooling for a long time, and the good long-wave transmissivity of the polyester sheet allows an efficient radiative energy loss.

It is not easy to predict *a priori* the effect of a ventilated chamber over the gas exchange rate of an enclosed plant. The changes in the concentration of atmosphere gases may have complex effects on many physiological processes (Bazot et al., 2008). Although many disturbances generated by the confined atmosphere act toward a rate decrease with respect to the undisturbed plant in open air, for example the reduction in the leaf-atmosphere gradient or in the radiation available (Nicolás et al., 2008), some other perturbations may have the opposite result. For example, in calm conditions, the artificial ventilation may well reduce the boundary layer thickness of the leaves inside the chamber compared with those outside, thus actually enhancing the gas exchange rate. The results of the sap velocity experiment proved that this hypothesis is not true for this chamber: the effect of long exposition to the enclosed environment always reduced the transpiration rate measured independently, and the reduction seems directly proportional to the closure duration (Figure 5.2a and b). Consequently, the chamber should be closed during the shortest time possible to avoid underestimation of water vapour, and - presumably - CO_2 fluxes.

Canopy conductance (G_c) of olive trees resulted affected by chamber closure to a very small degree, and only after a closure duration that exceeds by a considerable margin every measurement requirement (Figure 6.2). Therefore, the transpiration responded mainly to changes in the humidity gradient. Unfortunately, we had no independent measurement of CO_2 assimilation (as we had of transpiration) to explicitly prove that the overall change in the rate of CO_2 flux due to canopy enclosing is irrelevant. Nevertheless, the relative changes in the leaf/chamber CO_2 gradient occurring during a measurement are by far smaller than the ones in water vapour concentration, thus, as G_c is almost constant, the changes in assimilation (or respiration) rates induced by the chamber must be very small.

The optimal duration of the calculation window should be at the same time: a) long enough to allow an adequate absolute gas concentration increase that minimise the error in the flux calculation; and b) short enough to induce the lesser possible nonlinearity in the gas concentration vs. time. The nonlinearity in the gas concentration vs. time within a single closure operation can be taken as a measure of the disturbance inflicted to the system. In a given chamber, this nonlinearity depends not only on the closure time, but also on the actual gas exchange rate of the canopy under study. The analysis conducted comparing quadratic and linear regression fits under very different conditions suggest that nonlinearity is almost unavoidable at high gas exchange rate, but still it is extremely small with 180s closing time. We established that 100s (30s of lag and stirring time plus 70 second of calculation window) is an optimal closure time for the chamber presented here.

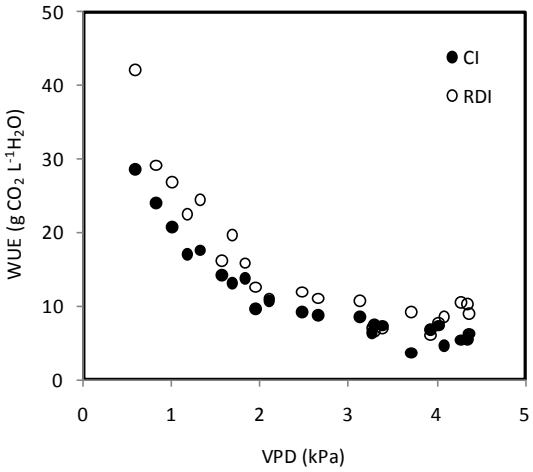

Figure 8.2. Relationship between Water Use Efficiency (WUE; g CO_2 L^{-1} H_20) and the vapour pressure deficit (VPD; kPa) of olive trees under different water status. Measurements taken on 6 September 2007. Black circles: well irrigated control (CI); white circles: water stressed (RDI treatment).

The relationship between hourly WUE and VPD in a well irrigated olive, presented in Figure 8.2 is very similar to those measured by Moriana et al. (2002) with a leaf cuvette and by Testi et al. (2008) with eddy covariance measurements. The WUE in the RDI tree was higher than the one of CI: stomatal closure (Figure 7.2c) under stress is a behaviour aimed to reduce the cost of carbon in terms of water used.

5. Conclusions

1) This work demonstrated that it is possible to measure the CO_2 and water vapour exchange rates of whole canopies up to 20-30 m^3 by a large closed chamber of the transient-state type.

2) The large chamber volume reduced the impact of the main sources of error in calculating the gas exchange flux in this type of chambers, namely: leakage, adsorption, canopy, air heating, and leaf/air gradient reduction.

3) Field tests showed that this chamber imposes minimum disturbance on the canopy environment, and that no appreciable alteration in canopy conductance is generated during standard measurements.

4) The minimum time required before the measurements of gas concentration can be used to calculate the gas exchange rate has been estimated in 30 s (lag and mixing time). The optimal duration for a measurement period with minimum disturbance on the canopy was estimated in 70 s.

5) The concurrent sap-flow measurements allowed to quantify the effect of the chamber closure on the transpiration rate. Enclosing the canopy reduced slightly the sap velocity, proportionally to the closing duration. Under normal measurement condition, with closure durations between 100 and 180 s, the transpiration at the time of re-opening should be reduced by less than 5%. It should be emphasised that this underestimation is only a potential disturbance; it will not affect the gas exchange measurements when the calculations are made with the quadratic method (where the rate is taken from the slope at the beginning of the calculation window).

6) The field measurements on trees in different water conditions yielded results that are in accordance with the literature.

7) The minimal effects that the chamber generated over the physiological processes of the enclosed tree indicate that this chamber design is suitable for accurate measures of gas exchange at tree level in almost all the cultivated fruit trees.

Acknowledgements

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References

Bazot S, Blum H, Robin C. 2008. Nitrogen rhizodeposition assessed by a 15NH3 shoot pulse-labelling of Lolium perenne L. grown on soil exposed to 9 years of CO2 enrichment. Environmental and Experimental Botany 63, 410–415.

Denmead OT. 1984. Plant physiological methods for studying evapotranspiration:problems of telling the forest from the trees. Agricultural Water Management 8(1–3), 167–189.

Denmead OT, Dunin FX, Wong SC, Greenwood EAN. 1993. Measuring water use efficiency of Eucalypt trees with chambers and micrometeorological techniques. Journal of Hydrology 150 (2–4), 649–664.

Dugas WA, Fitschen LJ, Held AA, Matthias AD, Reicosky DC, Steduto P, Steiner JL. 1991. Bowen ratio, eddy correlation, and portable chamber measurements of sensible and latent heat flux over irrigated spring wheat. Agricultural and Forest Meteorology 56, 1–20.

Dragoni D, Lakso AN, Piccioni RM. 2005. Transpiration of apple trees in a humid climate using heat pulse sap flow gauges calibrated with whole-canopy gas exchange chambers. Agricultural and Forest Meteorology 130 (1–2), 85–94.

Grau A. 1995. A closed chamber technique for field measurement of gas exchange of forage canopies. New Zealand Journal of Agricultural Research 38, 71–77.

Greenwood EAN, Beresford JD, Bartle JR. 1981. Evaporation from vegetation in landscapes developing secondary salinity using the ventilated-chamber technique. III. Evaporation from a Pinus radiata tree and the surrounding pasture in an agroforestry plantation. Journal of Hydrology 50, 155–166.

Held AA, Steduto P, Orgaz F, Matista A, Hsiao TC. 1990. Bowen ratio/energy balance technique for estimating crop net CO2 assimilation, and comparison with a canopy chamber. Theoretical and Applied Climatology 42, 203–213.

Jarvis PG, Catsky J, Eckart TE, Koch D, Koller D. 1971. General principles of gasometric methods and the main aspects of installation design. In: Sestak Z, Catsky J, Jarvis PG. (Eds.), Plant Photosynthesis Production: Manual of Methods. Dr. W. Junk N. V. Publishers, The Hague, pp. 49–110.

Leuning R, Foster IJ. 1990. Estimation of transpiration by single trees: comparison of a ventilated chamber, leaf energy budgets and a combination equation. Agricultural and Forest Meteorology 51 (1), 63–86.

McLeod MK, Daniel H, Faulkner R, Murison R. 2004. Evaluation of a enclosed portable chamber to measure crop and pasture actual evapotranspiration at small scale. Agricultural Water Management 67, 15–34.

McPherson HG, Green AE, Rollinson PL. 1983. The measurement, within seconds, of apparent photosynthetic rates using a portable instrument. Photosynthetica 17, 395–406.

Meyer WS, Reicosky DC, Barrs HD, Shell GSG. 1987. A portable chamber for measuring canopy gas-exchange of crops subject to different root zone conditions. Agronomy Journal 79, 181–184.

Moriana A, Villalobos FJ, Fereres E. 2002. Stomatal and photosynthetic responses of olive leaves to water deficits. Plant, Cell and Environment 25, 395–405.

Nicolás E, Barradas VL, Ortuño MF, Navarro A, Torrecillas, A, Alarcón JJ. 2008. Environmental and stomatal control of transpiration, canopy conductance and

decoupling coefficient in young lemon trees under shading net. Environmental and Experimental Botany 63, 200–206.

Orgaz F, Villalobos FJ, Testi L, Fereres E. 2007. Amodel of daily mean canopy conductance for calculating transpiration of olive canopies. Functional Plant Biology 34, 178–188.

Pickering NB, Jones JW, Boote KJ. 1993. Evaluation of portable chamber technique for measuring canopy gas exchange by crops. Agricultural and Forest Meteorology 63, 239–254.

Reicosky DC, Peter DB. 1977. A portable chamber for rapid evapotranspiration measurements on field plots. Agronomy Journal 69, 729–732.

Reicosky DC, Sharrat BS, Ljungkull JE, Barker D.G., 1983. Comparison of alfalfa evapotranspiration measured by weighing lysimeter and portable chamber. Agricultural Meteorology 28, 205–211.

Reicosky DC, Wagner SW, Devine OJ. 1990. Methods of calculating carbon dioxide exchange rates for maize and soybean using a Portable Field Chamber. Photosynthetica 24 (1), 22–38.

Reicosky DC. 1990a. Canopy gas exchange in the field: closed chambers. Remote Sensing Reviews 5 (1), 163–177.

Reicosky DC. 1990b. A portable chamber for evapotranspiration measurements and irrigation scheduling. Acta Horticulturae 278, 455–461.

Steduto P, Cetinkökü Ö, Albrizio R, Kanber R. 2002. Automated closed-system canopy-chamber for continuous field-crop monitoring of CO2 and H2O fluxes. Agricultural and Forest Meteorology 111, 171–186.

Testi L, Orgaz F, Villalobos FJ. 2008. Carbon exchange and water use efficiency of a growing, irrigated olive orchard. Environmental and Experimental Botany 63, 168–177.

Testi L, Villalobos FJ. 2009. New approach for measuring low sap velocities in trees. Agricultural and Forest Meteorology 49, 730–734.

Villalobos FJ, Orgaz F, Testi L, Fereres E. 2000. Measurement and modeling of evapotranspiration of olive (Olea europaea L.) orchards. European Journal of Agronomy 13, 155–163.

Wagner SW, Reicosky DC. 1992. Closed-chamber effects on leaf temperature, canopy photosynthesis, and evapotranspiration. Agronomy Journal 84 (4), 731–738.

Wagner SW, Reicosky DC, Saamuel Alessi R. 1997. Regression models for calculating gas fluxes measured with a closed chamber. Agronomy Journal 89, 279–284.

Chapter 2: Effects of water supply on carbon and water exchange of olive trees

Abstract

Little information is available on carbon exchange of olive orchards despite their agronomical and ecological importance. Measurements of CO_2 and water vapour exchange were performed during 2006 and 2007 with large closed chambers in an olive orchard in Cordoba (Spain) under two irrigation regimes, full and regulated deficit irrigation.

Canopy assimilation was higher for full (9.6-22.0 g CO_2 m^{-2} day^{-1}) than for deficit irrigation (8.0-19.4 g CO_2 m^{-2} day^{-1}). Water Use Efficiency typically decreased from maximum values around 30 g CO_2 L^{-1} soon after sunset to 2-7 g CO_2 L^{-1} in the afternoon when vapour pressure deficit typically exceeded 5 kPa. We estimate that Forty-five percent of gross assimilation was lost in respiration being root respiration less than 30% of total respiration. While water stress improved instantaneous Water Use Efficiency (WUE) only slightly, the effect was dramatic for daily values. The measurements allowed the calibration of a model coupling canopy conductance and assimilation that showed a seasonal variation in the parameters suggesting changes in the physiology of olive trees.

The improvement of WUE in olive trees under water stress supports the adoption of deficit irrigation in olive orchards although further research is required to prevent negative side effects.

1. Introduction

Olive (*Olea europaea* L.) is a very important crop in the Mediterranean area. Spain is the main world's producer of olive oil with more than 2.5 Mha of cultivated area. Irrigated olive area has increased from 200 000 ha to more than 400 000 in the past 15 years and has become the main irrigated crop in Spain. This conversion to irrigation has led to increased yield and reduced variability in oil production. However, most irrigated olive orchards have not enough water to meet the crop water requirement, thus demanding the development of deficit irrigation strategies (Iniesta et al., 2009).

One of the most promising deficit irrigation strategies is the regulated deficit irrigation (RDI) which consists of reducing water supply during periods when yield is less affected (Chalmers et al., 1981). Iniesta et al. (2009) compared RDI with continuous deficit irrigation in olive trees, concluding that RDI may be used to save significant amounts of irrigation water with moderate reductions in oil yield. From the work of Iniesta et al. (2009) and similar studies (e.g. Chalmers et al., 1981) it is clear that the water productivity of irrigation (yield/water applied) is higher with deficit irrigation. The improvement in the yield/water applied ratio may be due to different causes: 1) increased irrigation efficiency, which means that a larger fraction of irrigation water is used in evapotranspiration (ET), 2) improved partitioning of ET between transpiration and soil evaporation, 3) an increase in the water use efficiency (WUE) of leaves (the ratio of photosynthesis and transpiration) and 4) an increase in the harvest index, which is the fraction of carbohydrates directed to yield. The first and second causes depend on irrigation system characteristics and management while the third and the fourth are related to physiological responses to water stress.

In this work we have tested whether olive WUE is improved with water deficit. If this is so, the best strategy at a large scale for improving the productivity of irrigation water

would be to use deficit irrigation strategies. Otherwise the best alternative would be to reduce the irrigated area to keep enough irrigation water for maximum transpiration.

Although WUE is always a ratio of carbon gain to water loss, its meaning and relevance depends on the temporal and spatial scales of interest. In absence of water restrictions, WUE can be very stable at seasonal level (e.g. De Wit, 1958), but it shows ample diurnal variations (Zur and Jones, 1984; Testi et al, 2008).

The water use efficiency has been a key parameter in analyzing the response of plants to water stress, and thus, in breeding programs for "drought resistance", although using carbon isotope discrimination as a surrogate for WUE (Farquhar et al., 1989). Furthermore, WUE has been used extensively for calculating biomass accumulation in crop simulation models (e.g. Sinclair et al, 1984). The calculation of biomass from WUE is based on the following facts: a) as stomata are the common way shared by carbon and water at the same time, the resistances to carbon and water fluxes are linked and the relative flux is determined by the gradient of concentrations; and b) while the atmospheric concentration of carbon in air is relatively stable everywhere, the concentration of water vapour in air is widely changing in time and space. All other things being equal, WUE decreases when the atmospheric water demand increases. The biomass produced under well-watered conditions can thus be estimated with a simple function of the relatively stable WUE and the easy-to-obtain vapour pressure deficit (VPD) (Tanner and Sinclair, 1983). If WUE is unresponsive to water stress, or if its response can be predicted, WUE models could be still valid or easily adaptable to water stress conditions; unfortunately this assumptions are not yet confirmed.

In the present study we have used a closed chamber (Pérez-Priego et al. 2010) for evaluating the WUE of whole trees during a two-year study in an experiment where deficit irrigation strategies have been compared with a well irrigated control. The objectives of this work were to (a) characterize the diurnal and seasonal variations of

canopy photosynthesis (A_n), transpiration (E) and canopy conductance (G_c) of olive trees, and (b) to evaluate the effect of water stress on water use efficiency.

2. Material and Methods

2.1 Site description and Experimental Design

This study was conducted in a 4-ha drip-irrigated olive (Olea europaea L. cv. 'Arbequina') orchard planted in summer 1997 with a 3.5 x 7 m spacing, located in Cordoba, Southern Spain (37.85ºN, 4.80ºW, altitude 110 m). The climate in the area is Mediterranean, with an average annual rainfall of around 600 mm concentrated from autumn to spring, and potential reference ET of 1390 mm. The soil of the orchard is classified as a Typic Xerofluvent of sandy-loam texture and exceeding 1.5 m of depth.

The experiment was designed as a randomized complete block with three replicates and three treatments, although only two were included in this study. The first was control irrigation (CI) designed to meet the full evapotranspiration requirement. The second treatment was regulated deficit irrigation (RDI) which received 25% of the water applied to the control treatment with no irrigation applied during July and August. The amounts of water applied during the 2006 and 2007 seasons were 459 and 446 mm, respectively, for CI, and 120 and 63 mm, respectively, for RDI. Further details of this experiment may be found in Iniesta et al (2009).

2.2 Canopy gas exchange measurements

Two transitory-state chambers were built and arranged for simultaneous measurements of CO_2 and water vapour exchange in two trees during 2006 and 2007. The technique has been recently described and tested by Pérez-Priego et al. (2010) for measuring canopy gas exchange rate of olive trees. The chamber operates as a transitory-closed system (Steduto et al., 2002) and consists of a hexagonal prism with basal area of 10.4

m^2 and height of 4 m. The windows and roof, which are open and closed manually, are made of Llumar® "NRS90 clear" 75-µm thick polyester film, stretched and fixed to frames made of aluminium. The base of the chamber is sealed around the tree trunk through a 2-mm thick polyethylene sheet.

The measuring protocol was as follows: Just before measuring, the windows and top were closed and four 80-W fans were turned on. A vacuum pump took the air from intake points distributed through the chamber and then returned it to the chamber. A sample of 1 L min^{-1} of this air flow was sniffed using a small pump (model SP 270 EC, Schwarzer Precision GmbH, Essen, Germany) and diverted to a CO_2/H_2O infrared gas analyser (IRGA) (model LI-840, LI-COR Biosci., Lincoln, NE, USA) which measured gases concentrations at 1 Hz sampling rate; the output was recorded by a datalogger (model CR23X, Campbell Scientific, Logan, UT, USA). After each measurement, the fans were turned off and the chamber went back to the open position.

The rate of change of the concentration of CO_2 and H_2O is the apparent flux of net photosynthesis and transpiration, respectively. The calculations take into account the correction for air temperature and atmospheric pressure inside the chamber (Reicosky et al. 1990) and use the quadratic regression model proposed by Wagner et al. (1997) in order to reduce the error due to non linear gas concentration change inside closed systems.

The diurnal trends in CO_2 and water vapour exchange were measured at 30-min intervals on five dates from August to December 2006 and four from July to October 2007 in one tree from the control and one from the RDI treatment (Table 1.3).

Measurements of the fluxes during the night were collected on 5 October 2006.

2.3 Auxiliary measurements

An infrared thermometer with a 52º field of view (model IRTS-P, Apogee, UT, USA) was mounted on the centre of the chamber top, facing downwards, to measure the temperature of the canopy. The temperature inside chambers of the transient state type tends to rapidly increase after closure; the canopy temperature measurements ensured that the temperature of the leaves never surpassed the free air temperature of more than 1.5 ºC during the closing periods (which never exceeded 3 min.).

The plant leaf area (PLA) of the trees was measured every 2 months using a plant canopy analyzer (model LAI2000, LI-COR, Lincoln, NE, USA) following the recommendations of Iniesta et al. (2009). A weather station, located less than 300 m from the experimental plot provided meteorological data (vapour pressure deficit, air temperature, precipitation, solar radiation and wind speed).

Leaf water potential (Ψ) was measured at midday with a Scholander's pressure chamber (Soil Moisture Corp., Santa Barbara, CA, USA) in two sunny leaves per tree.

2.4 Calculations

Canopy conductance for CO_2 exchange (G_c) was calculated by inverting the Penman-Monteith Equation assuming negligible aerodynamic resistance (e.g. Villalobos et al. 2000, Orgaz et al. 2007):

$$G_c = \frac{P\,E}{1.56\,D} \tag{1}$$

Where P is atmospheric pressure (kPa), D the vapour pressure deficit (kPa), E the transpiration rate (mol m^{-2} s^{-1}) and 1.56 is the ratio between the diffusion coefficients of

CO_2 and H_2O in air (0.16 and 0.25 cm^2 s^{-1}, respectively). Diurnal canopy conductance for CO_2 was calculated using Eq. 1 with average values of E and D during the daytime.

The transpiration model proposed by Orgaz et al. (2007) for olive trees was used to calculate potential daily transpiration of the trees. The model uses the daily fraction of intercepted PAR (Q_d) and a linear function of average daytime temperature. The amount of radiation intercepted by the trees was calculated by a simplified PAR interception model (Orgaz et al. 2007).

Potential transpiration estimated with the model (E_p) reproduced well the daytime integrals of transpiration rates measured with the chamber under conditions of good water supply (see below). Therefore, the relative transpiration defined as E/E_p was used as a potential transpiration index to evaluate the water status of the trees during the experiment. Reference ET was calculated using the Penman-Monteith FAO equation (Allen et al., 1998).

2.5 Model of canopy conductance

We used the model of Leuning (1995) to relate canopy conductance (G_c) to canopy net assimilation (A):

$$G_c = G_0 + b\frac{A}{C_s\left(1+\frac{D}{D_0}\right)} \quad (2)$$

Where G_0 is canopy conductance when A is zero, C_s is average CO_2 concentration at the leaf surface and b and D_0 are empirical coefficients. The three parameters of the model (G_0, b and D_0) were fitted using the Box's Complex optimization procedure (Box, 1965).

3. Results

3.1 Environmental conditions and water stress

The measurements were performed under clear sky conditions with a wide range of diurnal average temperatures (12.5-32.5ºC) and vapour pressure deficits (0.65-4.00 kPa). Reference evapotranspiration varied from 1.1 (December 2006) to 7.1 mm day^{-1} (August 2006) while wind speed was low to moderate (1.5-3.9 m s^{-1}) (Table 1.3).

Leaf water potential was much lower in the RDI than in the control tree. For instance, in summer 2006 the RDI tree showed values of -3.6 MPa while the control reached only -1.65 MPa (Table 2.3).

The ratio of transpiration and potential transpiration (E/E_p) indicated that water stress in the RDI tree was severe in August 2006 (0.27-0.39), September 2006 (0.34), August 2007 (0.66), September 2007 (0.42) and October 2007 (0.48), and nil in October 2006, December 2006 and July 2007 with values of E/E_p greater than 1 (Table 3.3). The control tree showed moderate water stress during August 2006 (E/E_p=0.57-0.76) (Table 3.3).

Plant leaf area during the experiment ranged from 27 to 43 m^2 in the control tree and 26-to 31 m^2 in the RDI (Table 2.3), which correspond to values of LAI of 1.1 to 1.75 and 0.7 to 1.25, respectively.

Pruning was performed in January 2007 reducing the difference in plant leaf area between the two trees during 2007 (Table 1.3).

Table 1.3. Environmental conditions for the days of measurements with chambers. VPD: vapour pressure deficit; air T: average air temperature; ET_0: reference evapotranspiration; Rs: global radiation.

Date	VPD (kPa)	air T (°C)	ET_0 (mm day^{-1})	Rs (MJ m^{-2})	wind (m s^{-1})	daylength (h)
3-ago-06	2.54	30.07	7.10	24.64	3.93	13.88
24-ago-06	3.38	31.75	5.90	20.43	1.90	13.12
7-sep-06	2.86	29.85	4.50	17.36	1.56	12.55
5-oct-06	1.71	22.03	3.50	17.88	2.20	11.38
15-dic-06	0.65	12.45	1.10	7.33	2.33	9.36
5-jul-07	3.37	32.03	6.80	27.28	1.58	14.55
2-ago-07	4.00	32.41	6.90	24.48	2.14	13.91
6-sep-07	2.65	29.83	5.00	19.39	1.68	12.59
31-oct-07	1.28	16.73	2.80	13.50	3.36	10.36

Table 2.3. Canopy characteristics of the experimental trees in the measurement dates: volume (V), Leaf Area Density (LAD), Plant Leaf Area (PLA), fraction of daily PAR radiation interception (Q), potential transpiration obtained by model (E model) and midday leaf water potential (ψ).

CI	V (m^3)	LAD ($m^2\ m^{-3}$)	PLA ($m^2\ plant^{-1}$)	Q (n.d)	E_{model} ($mm\ day^{-1}$)	Ψ (MPa)
3-ago-06	25.10	1.53	38.40	0.50	2.91	-1.6
24-ago-06	25.60	1.45	37.12	0.51	2.61	-1.6
7-sep-06	26.15	1.60	41.84	0.52	2.12	-1.44
5-oct-06	26.05	1.65	42.98	0.55	1.68	-0.92
15-dic-06	26.10	1.60	41.76	0.65	0.46	-0.50
5-jul-07	16.10	2.03	32.68	0.37	2.52	-0.92
2-ago-07	18.25	1.81	33.03	0.40	2.65	-0.95
6-sep-07	19.68	1.52	29.91	0.43	2.06	-1.01
31-oct-07	25.22	1.08	27.24	0.56	0.93	-0.82
RDI	**V** (m^3)	**LAD** ($m^2\ m^{-3}$)	**PLA** ($m^2\ plant^{-1}$)	**Qd** (n.d)	**E_{model}** ($mm\ day^{-1}$)	**ψ** (MPa)
3-ago-06	17.20	1.50	25.80	0.37	2.26	-3.40
24-ago-06	17.50	1.60	28.00	0.38	1.96	-3.60
7-sep-06	17.70	1.62	28.67	0.39	1.74	-3.56
5-oct-06	17.42	1.55	27.00	0.42	1.31	-1.92
15-dic-06	17.60	1.60	28.16	0.51	0.36	-0.60
5-jul-07	14.06	2.19	30.79	0.33	2.29	-1.05
2-ago-07	15.25	1.88	28.67	0.35	2.44	-2.85
6-sep-07	16.75	1.50	25.12	0.38	1.87	-3.50
31-oct-07	19.09	0.90	17.18	0.45	0.75	-3.30

Table 3.3. Maximum, mean and total daytime net assimilation (A_n), transpiration (E), calculated ratio of transpiration to its potential value (E / E_{model}), mean conductance (G_c) and Water Use Efficiency (WUE) in the days of measurement.

CI	A_n max (μmol m^{-2} s^{-1})	A_n mean (μmol m^{-2} s^{-1})	A_n daytime (g CO_2 m^{-2})	E (L m^{-2})	E/E_{model} (n.d.)	G_c (mmol m^{-2} s^{-1})	WUE (g CO_2 L^{-1})
3-ago-06	10.86	6.20	13.64	1.67	0.57	47.5	8.16
24-ago-06	12.45	5.61	11.65	1.98	0.76	44.7	5.89
7-sep-06	12.22	6.37	12.67	2.09	0.99	58.4	6.05
5-oct-06	15.00	10.18	18.35	1.93	1.15	99.4	9.50
15-dic-06	11.26	6.47	9.59	0.54	1.19	88.9	17.61
5-jul-07	11.37	7.69	17.72	2.70	1.07	55.2	6.55
2-ago-07	14.76	9.39	20.69	3.29	1.24	59.3	6.29
6-sep-07	16.19	11.01	21.96	2.40	1.16	72.1	9.16
31-oct-07	14.80	11.25	18.46	1.17	1.25	88.4	15.78
RDI	**A_n max** (μmol m^{-2} s^{-1})	**A_n mean** (μmol m^{-2} s^{-1})	**A_n daytime** (g CO_2 m^{-2})	**E** (L m^{-2})	**E/E_{model}** (n.d.)	**G_c** (mmol m^{-2} s^{-1})	**WUE** (g CO_2 L^{-1})
3-ago-06	7.37	3.27	7.18	0.61	0.27	17.3	11.83
24-ago-06	9.33	3.87	8.04	0.77	0.39	17.4	10.49
7-sep-06	6.63	2.54	5.04	0.59	0.34	16.5	8.50
5-oct-06	14.18	8.28	14.93	1.51	1.15	77.8	9.86
15-dic-06	9.91	5.69	8.44	0.51	1.40	84.0	16.60
5-jul-07	13.07	8.40	19.37	2.78	1.22	56.8	6.96
2-ago-07	11.11	6.19	13.64	1.61	0.66	29.0	8.46
6-sep-07	8.40	5.08	10.14	0.79	0.42	23.7	12.82
31-oct-07	7.67	4.87	7.99	0.36	0.48	27.2	22.15

3.2 Canopy conductance

The diurnal course of canopy conductance presented a maximum during the early morning (8:00-9:00 h GMT) and then decreased until sunset. As an example the values of 2 August 2007 are presented in Fig. 1.3 showing maximum values of 160 and 119 mmol m^{-2} s^{-1} for the control and the deficit tree, respectively. There is a clear effect of water deficit on G_c as the deficit tree had values around 50% of those of the control tree for most of the day. Similar curves were observed in all dates although the differences between the control and the deficit tree were almost nil when the transpiration index was similar in the two treatments.

Average daytime conductance varied from 94 to 190 mmol m^{-2} s^{-1} for the control and between 39 and 168 mmol m^{-2} s^{-1} for the RDI tree with no significant differences in October 2006, December 2006 and July 2007 (Table 3.3).

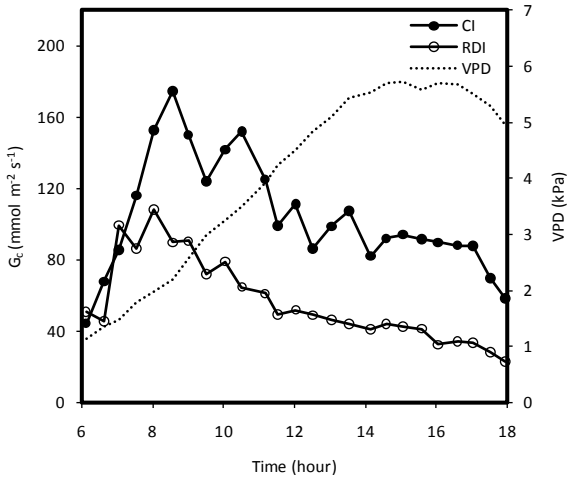

Figure 1.3. Diurnal time course of canopy conductance (G_c) for 2 August 2007 in control treatment (CI) and regulated deficit irrigation (RDI). Also shown the vapour pressure deficit (VPD).

3.3 Transpiration

Tree transpiration was maximum in the afternoon (13:00-14:00 h) for the control tree and showed a wide plateau in the RDI treatment. The daily course of transpiration in 6 September 2007, during the period when water stress was most severe in RDI, is shown as an example in Fig. 2.3. Transpiration increased from almost zero at sunrise to values close to 0.3 mm h^{-1} around 14:00 h in the control tree. The variation was less pronounced in the RDI reaching a maximum value of 0.07 mm h^{-1} and keeping similar values during most of the daytime.

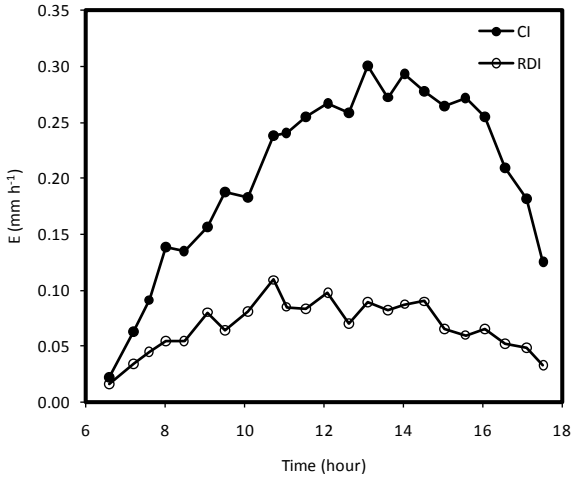

Figure 2.3. Diurnal time course of transpiration (E) for 6-sep-2007 in control treatment (CI) and regulated deficit irrigation (RDI).

Daily transpiration ranged from 0.5 to 3.3 mm day^{-1} in the control tree and between 0.4 and 2.8 mm day^{-1} in the RDI tree (Table 3.3) while ET_o varied from 1.1 to 7.1 mm day^{-1} (Table 1.3). Differences in transpiration between the two treatments occurred in the same dates as already indicated for canopy conductance.

3.4 Tree net assimilation

Aboveground net assimilation peaked in the early morning (8:00-9:00 GMT) with maximum values between 10.9 (3 August 2006) and 16.2 μmol CO_2 m^{-2} s^{-1} (September 2007) in the control treatment, and between 6.6 and 14.2 μmol CO_2 m^{-2} s^{-1} in the RDI treatment (Table 3.3). The diurnal time courses of assimilation for several dates are shown in Fig. 3.3 for the control tree. Values of A_n in October were clearly higher in the control during most of the day, while maximum values in July and December were similar for both treatments.

Daily total assimilation varied from 9.6 (December 2006) to 22.0 g CO_2 day^{-1} (September 2007) in the control and from 5.0 (September 2006) to 19.4 g CO_2 day^{-1} (July 2007) in the RDI tree (Table 3.3).

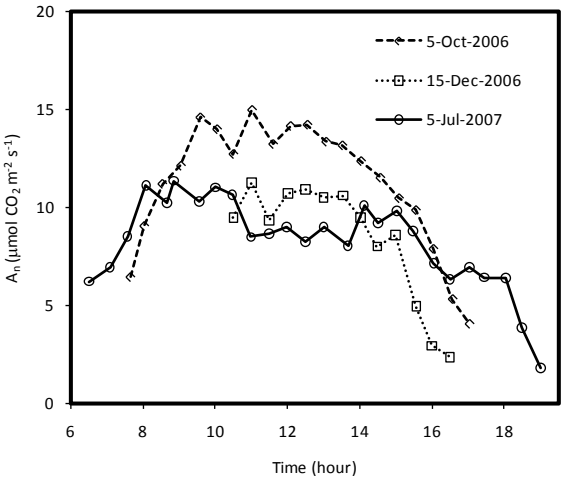

Figure 3.3. Diurnal time course of net assimilation (A_n) for 3 dates (5/10/06, 15/12/06, and 5/7/07) in the control treatment.

3.5 Water-use efficiency

Instantaneous WUE declined from sunrise to sunset with slightly higher values in the RDI tree (Fig. 4.3). The variation of WUE during the daytime was large with values as high as 30 g CO_2 L^{-1} at the beginning of the day (Fig. 4.3, RDI), decreasing down to 2.7 g CO_2 L^{-1} at the end of the day (Fig. 4.3, Control). A small increase in WUE just before sunset could be observed in specific dates as shown in Fig. 4.3 for 6 September 2007.

Diurnal mean WUE varied from 5.9 to 17.6 g CO_2 L^{-1} for the control tree and from 7.0 to 22.2 g CO_2 L^{-1} for RDI (Table 3.3). Mean WUE was higher in RDI on all dates except when water stress was absent.

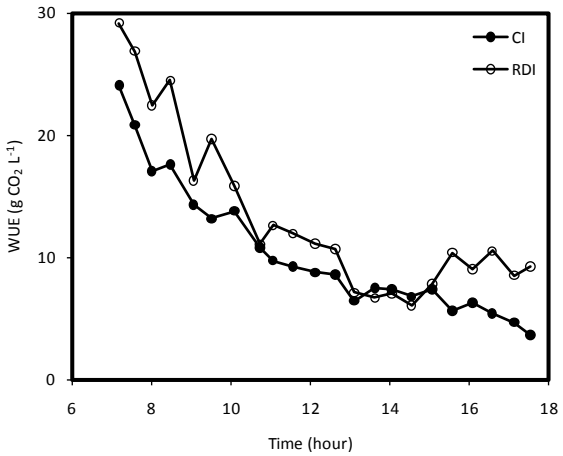

Figure 4.3. Diurnal time course of water use efficiency (WUE) for 6-sep-2007 in control treatment (CI) and regulated deficit irrigation (RDI).

3.6 Relation between assimilation and canopy conductance

The parameters of the conductance model (Eq. 3) varied widely for the different dates and the two treatments (Table 4.3). The value of G_0 ranged from 0.06 (minimum set at

the optimization procedure) to 12.6 mmol m^{-2} s^{-1} for the control tree and from 0.06 to 38.6 mmol m^{-2} s^{-1} for the RDI.

In general the parameter b was higher for the control tree than for RDI and the opposite occurred for parameter D_0.

Table 4.3. Parameters of the Leuning-Ball-Berry model at the canopy scale of olive trees cv 'Arbequina' based on chamber measurements.

	CI				**RDI**			
	G_0	**b**	**D_0**	**RMSE**	**G_0**	**b**	**D_0**	**RMSE**
	mol m^{-2} s^{-1}	n.d	kPa	mol m^{-2} s^{-1}	mol m^{-2} s^{-1}	n.d	kPa	mol m^{-2} s^{-1}
	x 10^{-3}			x 10^{-3}	x 10^{-3}			x 10^{-3}
3-Aug-06	0.06	4.18	11.08	7.95	1.09	3.08	21.08	7.05
24-Aug-06	11.15	10.07	0.97	1.92	1.35	3.30	21.54	11.09
7-Sep-06	7.44	4.95	3.45	10.13	9.42	3.43	1.82	6.28
5-Oct-06	0.06	6.25	2.57	7.82	12.18	4.31	4.60	18.14
15-Dic-06	2.12	8.02	0.93	9.04	38.59	11.98	0.24	12.24
5-Jul-07	5.06	3.49	9.78	5.96	7.05	2.71	13.79	3.97
2-Aug-07	2.63	2.67	19.71	5.83	2.88	2.36	25.95	5.71
6-Sep-07	12.63	2.76	11.20	5.96	0.06	2.39	27.02	4.23
31-Oct-07	4.87	2.98	18.95	9.04	13.08	8.84	0.28	13.59
All*	15.5	2.82	5.47	12.00	4.7	2.52	6.65	10.2

*excluding days with water deficit for the control and days with no water stress in the RDI.

4. Discussion

4.1 Net assimilation

Transient-state chambers as those used in this study have the main advantage of a minimal disturbance of the environment around the tree as compared with steady-state chambers (Perez-Priego et al., 2010) but they require to be operated on site limiting data collection, mainly during the night. Eddy covariance measurements of CO_2 exchange, which have been used extensively in recent years (e.g. Euroflux, Ameriflux projects, Falge et al. 2002), do not measure assimilation of the plant community but Net Ecosystem Exchange (NEE) and rely on strong assumptions for flux corrections.

These data are the first tree-level measurements of carbon and water exchange in large olive trees but may be compared to those collected by Testi et al. (2008) using eddy covariance that reported NEE values around 10 g CO_2 m^{-2} day^{-1} (4.5 μmol CO_2 m^{-2} s^{-1}) in summer with LAI close to 1.5, while soil CO_2 efflux was around 0.05 mg CO_2 m^{-2} s^{-1} (1.14 μmol CO_2 m^{-2} s^{-1}) implying an average net assimilation (excluding root respiration) during the daytime of 5.6 μmol CO_2 m^{-2} s^{-1}. Our summer data (Table 3.3) are higher in general, with a range of 5.6-11.0 μmol CO_2 m^{-2} s^{-1}. Higher values were observed in 2007, when LAI was lower due to pruning. The renewal of leaves caused by pruning may be the cause of this increased assimilation although other factors cannot be discarded. Total night-time respiration measured on 5 October 2006 was 5.58 g CO_2 m^{-2} thus the net exchange of the tree crown for 24-h in that day is 12.77 g CO_2 m^{-2} which is close to the average value based on eddy covariance (14.2 g CO_2 m^{-2}).

Maximum assimilation reached by the control tree was 16.2 μmol CO_2 m^{-2} s^{-1} (10:42 h, 6 September 2007) when LAI was 1.2. Data compiled by Falge et al. (2002) of Gross Primary Production and Ecosystem Respiration (R_{eco}) from Euroflux and Ameriflux sites for Mediterranean evergreen forests show a maximum value of GPP-R_{eco} of 12.9 μmol CO_2 m^{-2} s^{-1}. The maximum value found for olive is clearly below the range found

for C3 crops by Schulze et al. (1994) between 25 and 45 μmol CO_2 m^{-2} s^{-1}. If we take into account the low radiation interception of the control olive tree (43%) we may conclude that assimilation capacity of olives is within the range of C3 crops.

Radiation-use efficiency based on biomass accumulation has been reported for non-flowering olive trees as 1.3 g dry matter (MJ PAR)$^{-1}$ by Mariscal et al. (2000) and in fruit bearing trees by Villalobos et al. (2006) finding an average value of 0.86 g dry matter (MJ PAR)$^{-1}$. Comparing our chamber data with those values requires several assumptions. Daily root respiration can be assumed to be equal to average soil respiration measured by Testi et al. (2008) (0.05 mg CO_2 m^{-2} s^{-1}) which is reasonable due to the low organic carbon of the soil in this experiment (Testi et al. 2008). Assuming a root partitioning coefficient of 0.2 (Mariscal et al. 2000) and using a CO_2 to dry matter conversion factor of 0.6 we estimate for the control tree on 5 October 2006 a RUE of 0.9 g dry matter (MJ PAR)$^{-1}$ which is very close to the value found by Villalobos et al. (2006). A similar analysis leads to slightly higher RUE for the RDI tree (1.01 g dry matter (MJ PAR)$^{-1}$).

The analysis of the carbon balance on 5 October (Table 5.3) also shows that 45% of gross assimilation is lost in respiration and that root respiration is less than 30% of total respiration. Valentini et al. (2000) presented a general overview of the ratio of NEE and ecosystem respiration (R_{eco}) for different latitudes showing values around 1 in latitudes close to 40ºN which is close to our results in olive (NEE/R_{eco}=1.23).

Table 5.3. Carbon balance on 5 October 2006.

Component	units	CI	RDI
Night respiration	g CO_2 m^{-2}	5.58	4.38
Total night evaporation	mm	0.13	0.11
Total CO_2 exchange in 24 h	g CO_2 m^{-2}	12.77	10.55
Shoot respiration in 24 h*	g CO_2 m^{-2}	10.62	8.34
Root respiration in 24-h	g CO_2 m^{-2}	4.32**	3.39***
Gross photosynthesis	g CO_2 m^{-2}	33.3	26.7
RUE	g dry matter MJ	0.91	1.02
WUE in 24 h	g CO_2 L^{-1}	6.20	6.51

* assuming that daytime values are equal to night values
** taking 0.05 mg CO_2 m^{-2} s^{-1} for the 24-h period
*** assuming that root respiration is proportional to shoot respiration.

4.2 Water Use Efficiency

Water stress had a small effect on instantaneous WUE during the central hours of the day with differences typically below 10%, which is in contrast with Moriana et al. (2002) who did not find differences in instantaneous leaf WUE among different irrigation treatments in olive trees even under moderate water stress. Improved WUE under water deficit found in our experiment could be caused by reduced growth respiration while measurements of Moriana et al. (2002) were performed in fully expanded leaves where growth respiration would be almost nil.

However mean diurnal WUE (WUE_m) increased strongly under water stress reaching a 78% increase on 24 August 2006 (Table 3.3). This effect is explained by an increase in WUE during the morning, when VPD was lower and by the relative variation in stomatal opening in the RDI tree which reduces the contribution of high VPD to the daily transpiration as compared with the control.

Mathematically WUE_m is the transpiration-weighted average of WUE. Therefore for a decreasing diurnal pattern of WUE (Fig. 4.3), a decrease of transpiration in the afternoon (when WUE is low) leads to higher WUE_m as the contribution of the early morning (high WUE) is enhanced. The same analysis would apply when considering longer (seasonal) time scales when it is possible to impose a change in the relative distribution of transpiration by irrigation management. Reducing applied irrigation during summer would shift the relative transpiration distribution to conditions of higher WUE, thus improving seasonal WUE. Important implications are derived from this behavior. On the one hand, even if the physiological characteristics determining WUE are not affected by water stress, mean daily WUE may change if the pattern of stomatal opening is altered. On the other hand, long term WUE is not necessarily a result of physiological differences between plants, thus using carbon discrimination (Farquhar et al., 1989) in breeding programs for high intrinsic WUE should be taken with caution.

Furthermore, crop models of daily time step based on WUE for biomass accumulation (e.g. Cropsyst, Stöckle et al., 2003) would underestimate biomass production of water stressed crops if the relationship between WUE and VPD is calibrated using well irrigated crops.

Water Use Efficiency was inversely related to VPD (Fig. 5.3) as expected with values greater in general than those reported by Moriana et al. (2002) for sunny illuminated leaves, indicating a larger positive contribution of shaded leaves to overall canopy efficiency than the negative contribution of non-photosynthetic organs to net assimilation at the tree level.

The wide variation in the parameters of the conductance model (Table 4.3) that relate conductance and assimilation may indicate seasonal changes in leaf physiology. Testi et al. (2006) found a clear seasonal variation in the parameters of a canopy conductance model of olive trees, although the causes are still unknown.

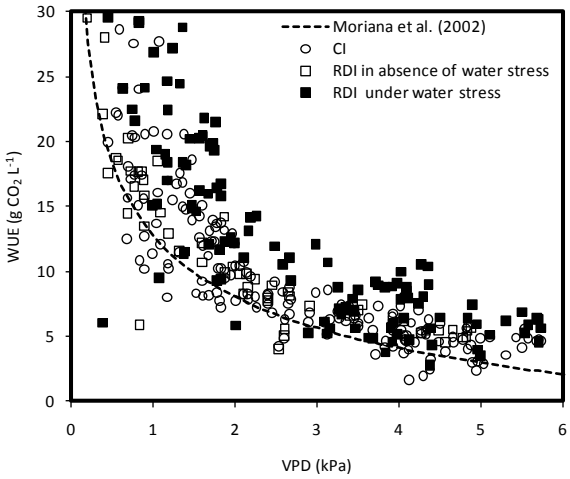

Figure 5.3. Instantaneous water use efficiency (WUE) plotted against vapour pressure deficit (VPD) for control irrigation (circles) and regulated deficit irrigation under and in absence of water stress (star and cross respectively). The line obtained by Moriana et al. (2002) with leaf measurements is also reported.

The relation between WUE and VPD found in this study follows the theoretical model based on the diffusion equations for assimilation and transpiration proposed by Tanner and Sinclair (1983). Alternatively, the conductance model may be the basis for understanding variations in WUE. By combining Eqs. 2 and 3 we may deduce:

$$WUE = \left(1 - \frac{G_0}{G_c}\right) \frac{C_s\ P}{1.56\ b} \quad \left(\frac{1}{D_0} + \frac{1}{D}\right) \tag{4}$$

This Equation shows that WUE will decrease when any of the parameters of the BBL model (G_0, b and D_0) increases or when stomata close (decrease in G_c). Also the asymptote of the WUE function is:

$$WUE_{asymp} = \frac{C_s\ P}{1.56\ b} \quad \frac{1}{D_0} \tag{5}$$

This value of WUE would be representative of high VPD conditions. According to Dewar (2002) the product of parameters b and D_0 is proportional to K, the hydraulic conductance between the bulk leaf and the guard cells, which decreases with leaf water potential. In physiological terms water deficit (low leaf water potential) reduces the hydraulic connection between the bulk leaf and the guard cells thus enhancing stomatal closure in response to high VPD. Therefore the increase in WUE with moderate water deficit found in this experiment may be explained by the reduction of the product bD_0.

The overall improvement of WUE in olive trees under water deficit supports the use of deficit irrigation strategies for maximizing biomass accumulation when water stress is concentrated in the summer (high VPD). Further research should define the limits of water deficits to avoid the negative side effects (reduced harvest index, leaf senescence, etc) that could neglect the benefits mentioned in the above.

Acknowledgements

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References

Allen RG, Pereira JS, Raes D, Smith M. 1998. Crop evapotranspiration : guidelines for computing crop water requirements. FAO irrigation and drainage paper, 56. Food and Agriculture Organization of the United Nations, Rome, 300 pp.

Box, M.J. 1965. A New Method of Constrained Optimization and a Comparison with other Methods. Computer Journal, 8(1): 42-52.

Chalmers DJ, Mitchell PD, and Vanheek L. 1981. Control of Peach-Tree Growth and Productivity by Regulated Water-Supply, Tree Density, and Summer Pruning. Journal of the American Society for Horticultural Science, 106(3): 307-312.

De Wit C. 1958. Transpiration and crop yields. Verslagen van Landbouwkundige Onderzoekinge, 64(6): 1-88.

Dewar RC. 2002. The Ball-Berry-Leuning and Tardieu-Davies stomatal models: synthesis and extension within a spatially aggregated picture of guard cell function. Plant, Cell and Environment, 25(11), 1383-1398.

Falge E, Baldocchi D, Tenhunen J, Aubinet M, Bakwin P, Berbigier P, Bernhofer C, Burba G, Clement R, Davis KJ, Elbers JA, Goldstein AH, Grelle A, Granier A, Gumundsson J, Hollinger D, Kowalski AS, Katul G, Law BE, Malhi Y, Meyers T, Monson RK, Munger JW, Oechel W, Paw U KT, Pilegaard K, Rannik U, Rebmann C, Suyker A, Valentini R, Wilson K, Wofsy S. 2002. Seasonality of ecosystem respiration and gross primary production as derived from FLUXNET measurements. Agricultural and Forest Meteorology 113(1-4): 53-74.

Farquhar G, Ehleringer J, Hubick K., 1989. Carbon isotope discrimination and photosynthesis. Annual Review of Plant Biology, 40(1): 503-537.

Iniesta F, Testi L, Orgaz F, Villalobos FJ. 2009. The effects of regulated and continuous deficit irrigation on the water use, growth and yield of olive trees. European Journal of Agronomy, 30(4): 258-265.

Leuning R. 1995. A Critical-Appraisal of a Combined Stomatal-Photosynthesis Model for C-3 Plants. Plant Cell and Environment, 18(4): 339-355.

Mariscal MJ, Orgaz F, Villalobos FJ. 2000. Radiation-use efficiency and dry matter partitioning of a young olive (Olea europaea) orchard. Tree Physiology, 20(1): 65-72.

Moriana A, Villalobos FJ, Fereres E. 2002. Stomatal and photosynthetic responses of olive (Olea europaea L.) leaves to water deficits. Plant Cell and Environment, 25(3): 395-405.

Orgaz F, Villalobos FJ, Testi L, Fereres E. 2007. A model of daily mean canopy conductance for calculating transpiration of olive canopies. Functional Plant Biology, 34(3): 178-188.

Pérez-Priego O, Testi L, Orgaz F, Villalobos F. 2010. A large closed canopy chamber for measuring CO2 and water vapour exchange of whole trees. Environmental and Experimental Botany, 68(2): 131-138.

Reicosky DC, Wagner SW, Devine OJ. 1990. Methods of calculating carbon dioxide exchange rates for maize and soybean using a Portable Field Chamber. Photosynthetica. 24 (1) 22–38.

Schulze ED, Kelliher FM, Korner C, Lloyd J, Leuning R. 1994. Relationships among maximum stomatal conductance, ecosystem surface conductance, carbon

assimilation rate, and plant nitrogen nutrition: a global ecology scaling exercise. Annual Review of Ecology and Systematics, 25:629-660.

Sinclair T, Tanner C, Bennett J. 1984. Water-use efficiency in crop production. Bioscience, 34(1): 36-40.

Steduto P, Çetinkökü Ö, Albrizio R, Kanber R. 2002. Automated closed-system canopy-chamber for continuous field-crop monitoring of CO2 and H2O fluxes. Agricultural and Forest Meteorology, 111(3): 171-186.

Stöckle C, Donatelli M, Nelson R. 2003. CropSyst, a cropping systems simulation model. European Journal of Agronomy, 18(3-4): 289-307.

Tanner CB, Sinclair TR. 1983. Efficient water use in crop production: research or research? In: Taylor HM, Jordan WR, Sinclair TR (Eds), Limitations to Efficient Water Use in Crop Production. ASA, CSSA, SSSA, Madison, Wisconsin, USA, pp. 1-27.

Testi L, Orgaz F, Villalobos FJ, 2006. Variations in bulk canopy conductance of an irrigated olive (Olea europaea L.) orchard. Environmental and Experimental Botany, 55(1-2): 15-28.

Testi L, Orgaz F, Villalobos FJ. 2008. Carbon exchange and water use efficiency of a growing, irrigated olive orchard. Environmental and Experimental Botany, 63(1-3): 168-177.

Valentini R, Matteucci G, Dolman AJ, Schulze ED, Rebmann C, Moors EJ, Granier A, Gross P, Jensen NO, Pilegaard K, Lindroth A, Grelle A, Bernhofer C, Grünwald T, Aubinet M, Ceulemans R, Kowalski AS, Vesala T, Rannik Ü, Berbigier P, Loustau D, Guomundsson J, Thorgeirsson H, Ibrom A, Morgenstern K, Clement

R, Moncrieff J, Montagnani L, Minerbi S, Jarvis PG. 2000. Respiration as the main determinant of carbon balance in European forests. Nature 404(6780): 861-865.

Villalobos FJ, Orgaz F, Testi L. and Fereres, E., 2000. Measurement and modeling of evapotranspiration of olive (Olea europaea L.) orchards. European Journal of Agronomy, 13(2-3): 155-163.

Villalobos FJ, Testi L, Hidalgo J, Pastor M, Orgaz F. 2006. Modelling potential growth and yield of olive (Olea europaea L.) canopies. European Journal of Agronomy, 24(4): 296-303.

Wagner SW, Reicosky DC, Saamuel Alessi R. 1997. Regression models for calculating gas fluxes measured with a closed chamber. Agronomy Journal 89 279–284.

Zur B, and Jones J. 1984. Diurnal changes in the instantaneous water use efficiency of a soybean crop. Agricultural and Forest Meteorology, 33(1): 41-51.

Chapter 3: Soil and Plant Respiration of an Olive Orchard

Abstract

- Respiration rates of spatially separable contributors to ecosystem respiration (R_e) such as soil (R_{soil}) and aboveground parts of the plant (R_{plant}) were measured with chamber-based techniques in an olive orchard in Córdoba (Spain).
- The measurements consisted in: I) R_{plant} and R_{soil} over 3 years in a drip-irrigated to assess the seasonal patterns; II) growth and maintenance components of R_{plant} for different organs in young trees using prolonged darkness periods and selective pruning; III) specific respiration of a large tree under different organ composition with successive pruning operations; IV) respiration response to temperature of woody and non-woody parts by stepwise defoliation of an adult tree.
- I) While R_{soil} was almost constant, R_{plant} varied proportionally to seasonal temperature. R_{soil} was the largest contributor to R_{eco} when T<20ºC. II) Approximately 30% of R_{plant} was growth and 70% maintenance respiration, whose dependence with temperature was empirically determined for different organs. III) R_{plant} was more closely related to leaf mass than plant mass or leaf area. IV) Respiration rates of non-woody organs accounted for 6% of total R_{plant} in T range of 17-35 ºC.
- R_{soil} was a large component of R_{eco} and was strongly limited by soil humidity. Leaves were the main contributors to aboveground R_{plant}. More research about growth respiration is required.

1. Introduction

Olive (*Olea europaea* L.) is one of the most significant agricultural systems in the Mediterranean area, and could play an important role in the biogeochemical cycle as a possible sink of greenhouse gases (GHGs). However, little information is available on the capacity of olive orchards for carbon sequestration (Villalobos *et al.*, 2006).

Carbon net balance at ecosystem level (NEP-net ecosystem production) is the net balance of CO_2 entering and leaving the ecosystem during a time period (Smith *et al.*, 2010). Therefore, it is the difference between gross primary production (GPP, the gross uptake of CO_2 due to photosynthesis) and total ecosystem respiration (R_{eco}). The later includes respiration of heterotrophic organisms (R_h) as well as autotrophic respiration (R_a) of the above- and belowground plant organs for maintenance and tissue construction (Rambal *et al.*, 2004). Net primary production (NPP) is the difference between GPP and R_a (Gifford, 2003). Therefore:

$$NEP = GPP - R_{eco} = GPP - R_a - R_h = NPP - R_h \quad (1)$$

The partitioning of NEP into its components and their relation with biotic and abiotic drivers is needed to understand the mechanics of the spatial and temporal variations in the carbon budget of the world biomes (Law *et al.*, 2002). There is a lack of basic information on respiration rates in photosynthetic and non-photosynthetic plant organs for assessing the carbon economy of a tree or stand. In this sense, carbon cycle models require parameters derived from the partitioning of canopy assimilation and ecosystem respiration (R_a and R_h) by using different approaches (Reichstein *et al.*, 2003; Owen *et al.*, 2007; Smith *et al.*, 2010).

Diurnal and seasonal variations of photosynthesis rates of leaves have been measured in olive trees with cuvette chambers (Angelopoulos, 1996; Moriana *et al.*, 2002; Diaz-Espejo *et al.*, 2006) and recently Pérez-Priego et al. (2010) quantified net assimilation of

olive at whole-plant level with canopy chambers large enough to enclose medium-size trees with crown volumes up to 30 m^3.

Plant respiration is a large, environmental sensitive component of the carbon balance and can consume more than 60% of the carbon fixed in photosynthesis (Ryan, 1994; Ryan *et al.*, 1996; Valentini *et al.*, 2000). Many studies suggest that respiration is a key process in explaining variations in ecosystem productivity and may play a major role in the C balance of some ecosystems (Ryan *et al.*, 1997; Cannell & Thornley, 2000; Thornley & Cannell, 2000; Valentini *et al.*, 2000). However, respiration in olive trees has been studied only at the leaf level by Diaz-Espejo et al. (2006) and no direct measurements at whole-plant scale have been tried yet. Reliable estimations at the stand level require information about the respiration rates of the crown components, namely leaves, fruits, stem and branches (Ryan *et al.*, 1996; Damesin *et al.*, 2002) . Unfortunately, respiration measurements are quite complicated to perform for soil and plant organs, and scaling procedures are not always straightforward (Damesin et al., 2002).

Functional models of plant respiration partition it into the part used for construction of new tissue and the one that is used for maintenance of existing cells (Ryan, 1994). Maintenance respiration (R_m) is strongly related to N content, biomass, volume or surface area (Cannell & Thornley, 2000) and increases with temperature (McCree, 1982). Growth respiration is proportional to growth rate, which is also dependent on temperature, but in a weaker and different way than is maintenance respiration (McCree, 1982). Therefore, the response of both respiration components to temperature depends largely on how the relative growth rate is affected by temperature (Bunce, 2007). The dependence of respiration on temperature is further complicated by the phenomenon of acclimation, of which many studies reported evidence (Atkin *et al.*, 2000; Atkin *et al.*, 2006; Baldocchi, 2008). The causes of acclimation to temperature in the respiration of mature leaves have been related directly to the availability of

carbohydrates, changes in leaf nitrogen content and photosynthetic capacity (Bunce, 2007). Hence, the effect of temperature on respiratory CO_2 loss for predicting plant growth has still to be largely assessed.

The objectives of this study were: 1) to partition soil and aboveground plant respiration of olive trees in the field; 2) to estimate growth and maintenance components of plant respiration for different organs; 3) to assess how the mass-specific respiration of a large tree varies under different organ composition and 4) to quantify the response of respiration to temperature for trunk and non-woody organs.

2. Materials and methods

2.1 Experimental site

The experiments (with the exception of experiment II, that made use of potted trees) were conducted in a 4-ha olive (*Olea europaea* L. 'Arbequina') orchard planted in summer 1997 with a 3.5 m x 7 m spacing. The orchard is located in Córdoba, southern Spain (37.85ºN, 4.8ºW, altitude 110 m) under Mediterranean climate. An automated weather station placed less than 300 m away from the experimental plot provided meteorological data during the study period (that spanned from August 2006 to November 2008 for the different experiments). Rainfalls in the area are concentrated during autumn and winter; summers are hot and completely dry. The orchard was drip irrigated using 7 emitters per tree (with a discharge rate of 4 L h^{-1} each) to ensure the avoidance of any water stress. The soil of the orchard is classified as a Typic Xerofluvent of sandy-loam texture, which depth exceeded 1.5 m. Weeds were controlled using herbicides.

2.2 Canopy Chamber

The canopy chamber used has been recently described and tested by Pérez-Priego et al. (2010). The chamber operates as a transitory-closed system and consists of a hexagonal prism with base of 10.4 m^2 and height of 4 m. Windows and tops are made of Llumar® "NRS90 clear" polyester film of 75 µm thickness, stretched and fixed to aluminium frames. The bottom is sealed around the tree trunk through a thick polyethylene panel; thus, fluxes from the soil are excluded. Revolving side and top windows allow quick opening and closure of the device. During the closure periods (lasting 1 to 3 min) a vacuum pump circulates the inner air through the sampling circuit and a sample is diverted to a CO_2 infrared gas analyser (IRGA, model LI-COR LI-820, Lincoln, NE, USA) which measures the concentration of CO_2 at a sampling rate of 1Hz; the output is recorded by a datalogger (model CR23X, Campbell Scientific, Logan, UT, USA). After each measurement the chamber goes back to the open position to minimise the interference over the tree environment.

2.3 Experiment I: Soil and Plant respiration

Respiration rates of spatially separable contributors to R_{eco} such as soil and aboveground parts of the plant were measured with chamber-based techniques. The objective of this experiment was to characterize spatial and temporal variability of both soil (R_{soil}) and plant (R_{plant}) respiration of an olive orchard over 3-year field data.

Measurements of soil CO_2 effluxes (R_{soil}) were performed between 8:00 and 11:00 GMT at bi-monthly intervals from August 2006 to November 2008 using a gas exchange closed system. Rings made of PVC (16 cm diameter and 6 cm height) were inserted into the soil surface (3 cm deep) in 8 permanent sampling points. The measurements of R_{soil} were made with a chamber, specifically manufactured in the IAS-CSIC laboratory to fit and seal the ring. The chamber was coupled to an IRGA (model LI-COR LI-820, Lincoln, NE, USA) for measuring the variation of CO_2 concentrations

with time; the output was recorded by a datalogger (model CR23X, Campbell Scientific, Logan, UT, USA). The chamber with a volume of 0.007 m^3 was internally ventilated and equipped with a thermistor to record the air temperature. For each measurement the water content in the first 7.5 cm layer of soil was measured by a Time Domain Reflectometry apparatus (MiniTrase System, SoilMoisture Equipment Corp., Santa Barbara, CA, USA). The measurements were distributed in two main zones; 4 sampling points were located in permanent wetted areas close to the drippers (along the trees row) and the remaining 4 sampling points in the dry soil in the inter-rows alleys.

The canopy chamber was installed in the field for measuring night-time plant respiration (R_{plant}) on 10 October 2006, 15 December 2006, 31 October 2007, 12 May 2008 and 17 July 2008. Leaf area of trees was determined indirectly by measuring canopy transmissivity using a LAI-2000 canopy analyzer (LI-COR Lincoln, NE, USA). In the case of 12 May 2008 and 17 July 2008, leaf area was measured destructively with an electronic planimeter (Li-Cor. LI-3100, LI-COR, Inc., Lincoln, NB, USA) because these measurements were extended for further studies (experiment III and IV).

2.4 Experiment II: Small trees in prolonged dark condition

This experiment was carried out in the Instituto de Agricultura Sostenible (IAS) of Córdoba from 1 to 7 August 2008, and was aimed at quantifying the contribution of growth and maintenance components to total respiration in young olive trees. The experiment was designed to assess the temperature response of maintenance respiration as well, and its partition into specific respiration coefficients for different organs (fruit, leaves and fine branches). The experimental approach relies on the theoretical assumption that growth respiration is almost nil after a prolonged darkness period (McCree & Silsbury, 1978; Gifford, 2003).

Four three-year old olive trees (*Olea europaea* L. 'Arbequina'), growing in a soil peat mixture in 50 L pots, were placed during 6 days in a smaller (8 m^3) version of the

transitory closed chamber system described in Pérez-Priego et al. (2010). The sides of the chamber, including the top, were carefully covered with aluminium foil to maintain the trees in total and permanent darkness while avoiding overheating from direct sun exposure. The inner atmosphere was kept in equilibrium with the outside environment by a ventilation system when the opaque chamber was not operated. Two respiration data sets were collected, the first 6-8 h after placing the trees in the chamber and the second after a standardised time (48 h) of continuous darkness, when the maintenance respiration rate (R_m) is theoretically achieved (Gifford, 2003). Afterwards, while plants were still kept in darkness, measurements of CO_2 exchange were made over 4 days between 9:00 and 13:00 GMT. At the end of each measurement day a class of organs of the plants was removed (fruits, leaves and stems). The CO_2 efflux from the bare pots remaining at the end (0.8 µmol CO_2 s^{-1}) was discounted. The R_m at standard temperature (25 ±1 ºC) for fruits, leaves and stems was obtained as difference between measurements at that temperature in different days. The dry biomass of the removed organs was obtained after drying them at 70 ^oC for 24 h. The area of the leaf samples was measured with an electronic planimeter (model LI-3100, LI-COR, Inc., Lincoln, NB, USA). The leaf mass per unit leaf area (LMA; g m^{-2}) was calculated as the ratio between the dry mass of leaves and their fresh area.

2.5 Experiment III: Mass-specific respiration of a large tree under different organ composition

The objective of this experiment was to assess the mass-specific respiration (the total respiration per unit of dry mass) of the organs of a large tree. The canopy chamber was placed in the field for measuring respiration of a single tree from 12 to 15 May 2008. Successive pruning operations were performed and followed by respiration measurements, to obtain R_s in a tree with different organs fractions. The crown structure of the tree was supported by 3 main branches which were cut one each day followed by respiration measurements after sunset. Dry matter of removed leaves, fine branches and

trunk, leaf area and LMA were measured as in experiment II. The total plant leaf area of the tree was 67.47 m^2, equivalent to LAI 2.75 m^2 m^{-2} for the 3.5 x 7 m tree spacing. The above-ground dry biomass of the tree - measured destructively - was 72.9 kg, partitioned in fractions of 0.22 (22%) leaves and 0.78 (78%) non-leaf (wood and fine branches).

2.6 Experiment IV: Temperature response of trunk and non-woody organs

This experiment (which took place during 5 nights in July 2008) aimed to analyze the partition of respiration between woody and non-woody organs (leaves, fruits and fine branches) and to quantify their temperature response. Selected pruning operations of fruits, leaves and fine branches (with diameter smaller than 20 mm) were carried out in an adult olive tree during 5 mornings. Measurements of CO_2 efflux of the whole tree were performed 4 hours after sunset, and at the end of each night. The response of respiration to temperature of the bole of the tree was measured once the tree was completely defoliated. Non-woody respiration was calculated as the difference between whole-plant and trunk respiration. Dry matter of removed leaves, fine branches and trunk, leaf area and LMA were measured as in Experiments II and III. Plant leaf area was 70.31 m^2 and the initial aboveground dry biomass was 102.55 kg.

2.7 Temperature sensitivity

To quantify variations in respiration (R, µmol CO_2 m^{-2} s^{-1} or µmol CO_2 kg^{-1} s^{-1}) caused by variations in air temperature (T expressed in K) for data of experiments II and IV we used the modified Arrhenius equation proposed by Lloyd & Taylor (1994) for a reference respiration rate (R_{ref}) at the base temperature of 18ºC:

$$R = R_{ref} e^{E_0 \left(\frac{1}{301.15 - T_0} \right) \left(\frac{1}{T - T_0} \right)} \quad (2)$$

where the optimised parameters are R_{ref} (µmol CO_2 kg^{-1} s^{-1}), E_0 (K) and T_0 (K).

3. Results

3.1 Soil and plant respiration (Experiment I)

Total soil respiration (R_{soil}) varied only between 1.4 and 2.9 µmol CO_2 m^{-2} s^{-1} over the 8-33 ^oC air temperature interval; the average was 2.2 µmol CO_2 m^{-2} s^{-1} (Fig. 1.4). Soil humidity was source of a larger variation in soil CO_2 efflux. In periods without rainfalls, the volumetric soil water content in the area wetted by the irrigation drippers (14 % of the total surface) was kept steadily around 0.28 m^3 m^{-3} due to the high-frequency irrigations, while it was only 0.07 m^3 m^{-3} in the rest. Whereas respiration rates measured in the dry alleys presented a rather stable value around 1.5 µmol CO_2 m^{-2} s^{-1}, R_{soil} from the drippers' wet spots showed a wide range, spanning from 2.1 to 11.3 µmol CO_2 m^{-2} s^{-1}. High R_{soil} values - around 7 µmol CO_2 m^{-2} s^{-1} - were observed in winter time (December 2006) as well as in warmer periods (summer 2007 and 2008).

Aboveground plant respiration (R_{plant}) measured at different growing periods was proportional to air temperature (Fig. 1.4). Seasonally, R_{plant} measured in the same tree at different times of the growing period over 2006 varied from 0.7 µmol m^{-2} s^{-1} (at 14 ^oC; December 2006) to 3.5 µmol m^{-2} s^{-1} (at 24 ^oC; October 2006). R_{plant} measured in October 2007 in a different tree but at temperatures from 7 to 14 ^oC was as low as in December 2006 (0.4- 1.2 µmol m^{-2} s^{-1}). Although with an apparent scatter, R_{plant} measured in May and June 2008 at temperatures exceeding 17 ^oC was similar to that measured in October 2006 (1.5 – 3.9 µmol m^{-2} s^{-1}).

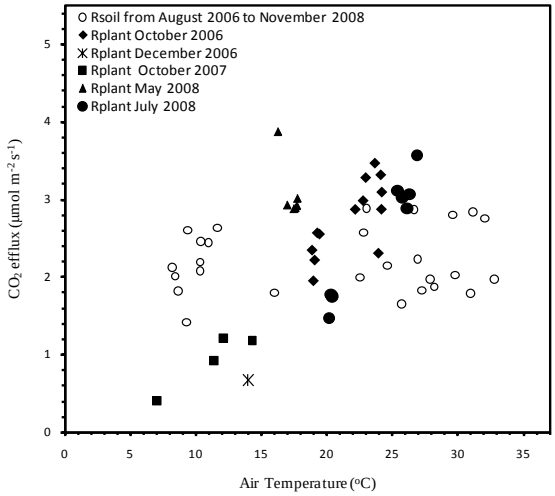

Figure 1.4. Response to temperature of average soil respiration (R_{soil}; White circles) and aboveground plant respiration (R_{plant}) measured in different dates (Experiment I).

3.2 Respiration of leaves, fruits, and stems in young olive trees (Experiment II)

Respiration rates measured 6-8 h after placing the trees in the chamber (with temperatures between 28 and 31 ºC) showed a mean value of 3.22 µmol CO_2 s^{-1} (2.39 µmol CO_2 kg^{-1} s^{-1} mass-specific). The average CO_2 efflux in the same temperature range after 48 h in continuous darkness was 2.27 µmol s^{-1} (1.69 µmol kg^{-1} s^{-1}), a reduction of 30% (Fig. 2.4). The CO_2 efflux of whole plants (fruit+leaf+stem) measured after 48 h in continuous darkness varied from 1.14 to 3.2 µmol CO_2 s^{-1} over the 24-33 ºC temperature range. Once fruits were removed, CO_2 efflux from leaves and stems varied from 0.58 (at 24.5 ºC) to 1.5 µmol CO_2 s^{-1} (at 31.5 ºC). Finally, CO_2 efflux from stems only ranged from 0.19 (at 24 ºC) to 0.56 (at 31.3 ºC) µmol CO_2 s^{-1} after 5 days in continuous darkness. The maintenance respiration R_m of plant parts calculated at temperature of 25 ±1 ºC and expressed as function of dry mass were: 1.85 µmol CO_2 kg^{-1} s^{-1} for leaves, 1.55 µmol CO_2 kg^{-1} s^{-1} for fruits and 0.57 µmol CO_2 kg^{-1} s^{-1} for stems (Table 1.4).

Table 1.4. Mass-specific maintenance respiration coefficients (μmol CO_2 kg^{-1} s^{-1}) at 25 ºC of whole plant and different organs in young olive trees, as measured in Experiment II.

	R_m calculated at 25 ±1°C (μmol CO_2 kg DM^{-1} s^{-1})	Dry Biomass (kg)	$f_{biomass}$	Relative contribution (%)
Whole plant	1.30	1.35	1.00	-
leaf	1.85	0.35	0.26	37.4
fruit	1.55	0.54	0.40	47.8
stem	0.57	0.45	0.34	14.8

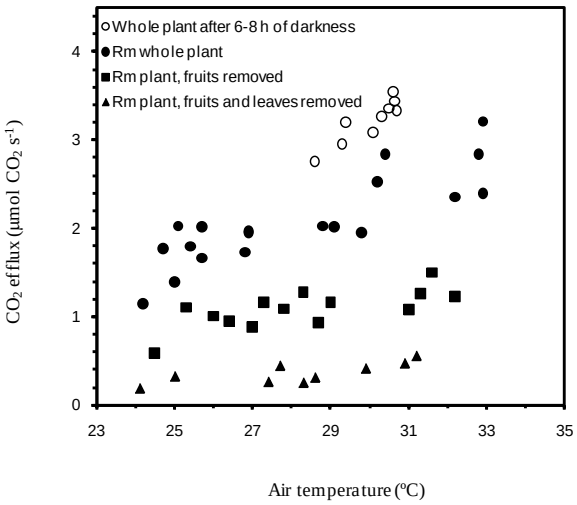

Figure 2.4. Total and maintenance respiration *vs.* temperature of 4 three-year-old olive trees in August 2008 (Experiment II). Total and maintenance respiration rates were measured after 6-8 h and 48 h of darkness, respectively.

The optimisation of the modified Arrhenius equation (Eq. 2) to mass-specific respiration yielded the results presented in Fig. 3.4. The total mass-specific R_m of the whole plants (fruit+leaf+stem, measured after 48 h in continuous darkness) varied from 0.85 to 2.38

µmol CO_2 kg^{-1} s^{-1} over the 24-33 ºC temperature range. The mass-specific respiration rates measured 6-8 h after placing the small trees in the chamber with temperatures between 28 and 31 ºC were always higher than R_m. The ratio between total and maintenance respiration in the 28-31 ºC temperature range was 1.42.

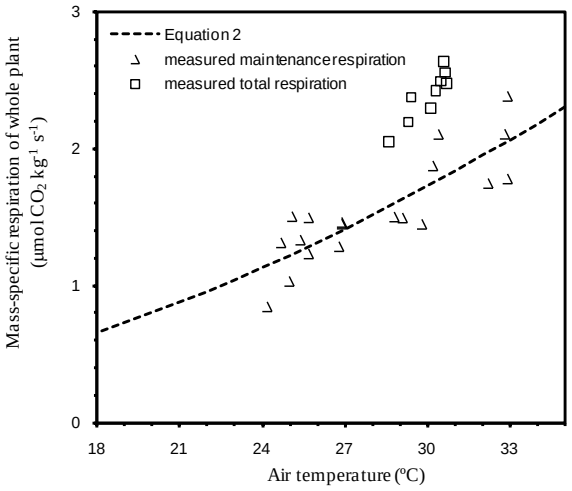

Figure 3.4. Mass-specific respiration rates of young olive trees after 6-8 h (plant respiration) and 48 h in continuous darkness (considered equivalent to maintenance respiration) measured in Experiment II. The line shows the best fit for Eq. 2.

3.3 Specific respiration in a large tree (Experiment III)

The pruning operations led to changes in the specific composition of the tree during the experiment III, with an increase in the wood fraction (from 0.67 to 0.76) and a decrease in leaves (from 0.22 to 0.17) and fine branches (from 0.11 to 0.07) (Table 2.4). The total mass-specific respiration measured at almost constant temperature (around 17 ºC) decreased from 0.95 to 0.79 µmol CO_2 kg^{-1} s^{-1} although no significant differences were found between the values taken in days 2 and 3 (Table 2.4). On the other hand, the respiration per unit leaf area increased from 1.02 to 1.28 µmol CO_2 m^{-2} s^{-1} with no

significant difference between days 1 and 2, but clearly higher on the third day. No significant differences were found over 3 days when plant respiration was expressed per unit of leaf mass.

Table 2.4. Specific respiration rates; Rspm (per unit plant mass),Rsla (per unit leaf area), Rslm (per unit leaf mass) and dry matter distribution among wood, fine branches and leaves of a large olive tree, Experiment III. Values are means (± SEM) where n = 6 measurements.

Days	Temperature (^oC)	R_{spm} (μmol CO_2 kg^{-1} s^{-1})	R_{sla} (μmol CO_2 m^{-2} s^{-1})	R_{slm} (μmol CO_2 kg^{-1} s^{-1})	LMA (g m^{-2})	f_{leaves}	$f_{fine\ branches}$	f_{wood}
1	17.53 ± 0.31	0.95 ± 0.02b	1.03 ± 0.02a	4.38 ± 0.08a	234.2	0.22	0.11	0.67
2	17.36 ± 0.24	0.8 ± 0.05a	1.02 ± 0.07a	4.27 ± 0.27a	238.5	0.19	0.09	0.72
3	16.07 ± 0.16	0.79 ± 0.06a	1.28 ± 0.09b	4.64 ± 0.34a	275.7	0.17	0.07	0.76

Values with different letters (a and b) indicate significant differences

(p<0.05,Tukey test) among days.

3.4 Temperature response of woody and non-woody organs (Experiment IV)

In Experiment IV, the initial fractions of biomass in woody and non-woody organs of the tree were 0.7 and 0.3, respectively. The 30% of biomass was composed of non-woody; 8% of fruits, 12% of leaves and 10% of fine branches. The CO_2 efflux measured in the bare tree structure (trunk and main branches) varied between 0.04 and 0.32 μmol CO_2 kg^{-1} s^{-1} in the temperature range of 17-35 ºC (Fig. 4.4a). The CO_2 efflux measured in non-woody organs varied between 0.73 and 2.67 μmol CO_2 kg^{-1} s^{-1} for the

temperature range 18-30 ºC (Fig. 4.4b). Respiration rates measured in the isolated trunk accounted for 6% of the value of the whole crown. The reference respiration rates (at 18 ºC) of trunk and non-woody organs were 0.06 and 0.95 µmol CO_2 kg^{-1} s^{-1}, respectively. The analysis of residuals error of equation 2 after optimisation showed unbiased distributions.

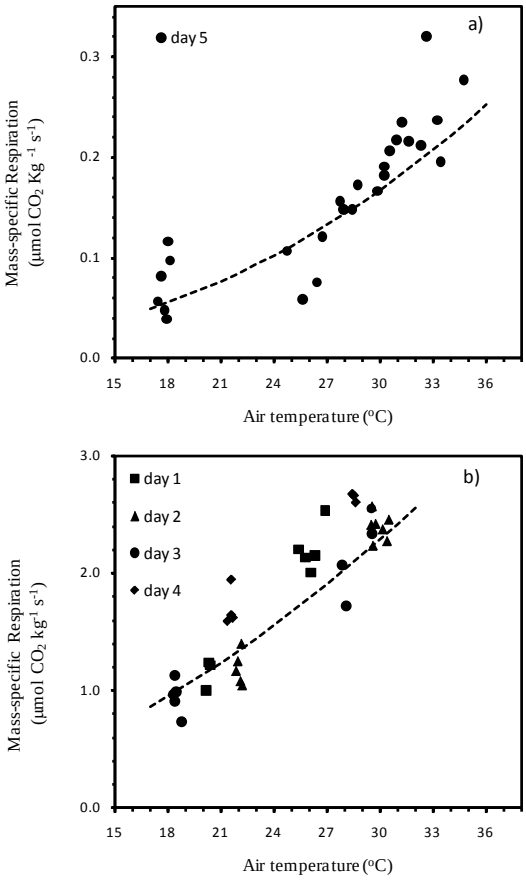

Figure 4.4. Temperature response of trunk (a) and non-woody organs (b) mass-specific respiration measured in the field along 5 nights in July 2008, Experiment IV. The response of respiration to temperature only in the bole of the tree was measured once the tree was completely defoliated.

4. Discussion

In experiment I, R_{soil} showed little or no dependence on the seasonal temperature cycle. Although R_{soil} was always higher in the wet zone than in non-wetted soil of the alley (6.0 vs. 1.5 µmol CO_2 m^{-2} s^{-1} in average, respectively) it did not increase after the rain events in the autumn, with still warm temperatures. This time-spatial pattern of soil respiration may be explained by the interaction of soil water content and root distribution: high root density and microbial activity in the wet zones only. These results are consistent with those reported by (Testi *et al.*, 2008) who obtained a similar spatial variation of R_{soil} in the same orchard (1.1 µmol CO_2 m^{-2} s^{-1} in the dry to 6.75 µmol CO_2 m^{-2} s^{-1} under drippers), with little change along the day despite a large variation in soil temperature. Assessing the autotrophic (roots) contribution to soil respiration is nevertheless very difficult (Hanson *et al.*, 2000).

Fig. 1.4 shows that R_{soil} was higher than R_{plant} when temperature was lower than 20 ºC; total R_{soil} was almost constant, whereas R_{plant} varied proportionally with air temperature. The estimated R_{eco} (as the sum of R_{soil} and R_{plant}) varied from 2.6 to 5.8 µmol CO_2 m^{-2} s^{-1} for the temperature range 7-27 ºC. These values are consistent with those measured with eddy covariance in the same orchard (Testi et al. 2008), ranging from 1.3 to 4.8 µmol CO_2 m^{-2} s^{-1} as the orchard was growing. Falge *et al.* (2002) using FLUXNET and AmeriFlux data found R_{eco} of Mediterranean evergreen systems oscillating between 0.9 and 7.4 µmol CO_2 m^{-2} s^{-1}, which is also consistent with our data.

We observed a strong variability in the mass- specific R_{plant} at a common measuring temperature (around 20ºC) among data obtained in October 2006, May 2008 and July 2008. Part of this variability may be due to the different contribution of the growth component of R_{plant} when the plant allocates most of the new assimilates to different organs in different seasons: shoots and flowers during the measurements taken in May and fruits in July and October.

Although there is not a rigorous division between growth and maintenance energy-requiring processes (Cannell & Thornley, 2000), experiment II showed that the respiration rates after 6-8 h of darkness were higher than those after 48 h and directly proportional to temperature. Upon the assumption that the latter are representative of maintenance metabolism, our results show that respiration in olive could be divided roughly in 30% for growth and 70% for maintenance. At 25ºC, the R_m of leaves is slightly lower (Fig. 2.4) than what reported in olive leaves by Díaz-Espejo *et al*. (2006) (0.8 μmol CO_2 m^{-2} s^{-1}) at the same temperature.

In fruits, R_m at 25 ºC was as high as in leaves (Fig. 2.4). Proietti *et al*. (1999) report a similar value at this stage of fruit development (≈ 2 μmol CO_2 kg^{-1} s^{-1}) in the final stage of development when the fruit stops growing. Growth respiration of fruits may be a large component during intense growth periods.

The results of experiment III (Table 2.4) over three nights in May 2008 (at almost constant temperature) confirms that total respiration rate is tightly related to dry mass composition and leaf area. Mass-specific plant respiration (R_{spm}) decreased significantly as the non-woody fraction was reduced. Respiration at leaf mass basis (R_{slm}) showed no significant differences among the 3 days.

Experiment IV confirms that the trunk and branches have much lower respiration rates than non-woody organs (an expected result, as wood contains mostly structural material with very little or null metabolic requirements). Respiration of the trunk accounted for 6 % of the total above-ground R_{plant} at 18 ºC. Expressed as volume-specific, at 15 ºC, our measurement of the respiration rate of the woody organs (0.04 μmol CO_2 kg^{-1} s^{-1}) equals 27 μmol CO_2 m^{-3} s^{-1}. For the sake of comparison (information in fruit crops is deficient), Ryan *et al.* (1997) gives values between 18 and 110 μmol CO_2 m^{-3} s^{-1} at 15 ºC across eight boreal forest stands. Damesin *et al.* (2002) found values seasonally ranging between 10.4 and 131.5 μmol CO_2 m^{-3} s^{-1} in 30-years-old *Fagus sylvatica* trees. Our measurement are consistent with these results, although on the low side, considering as

well that our trees were younger - thus with less fraction of structural wood. This may be the reason why the mass-specific R_{plant} of woody organs in Experiment IV (11-year-old trees) resulted lower than the R_m of the same component measured in Experiment II (3-year old plants). The small maintenance cost of structural wood indicates that aboveground R_{plant} is weakly affected by differences in woody biomass, and leaves are the main contributors to it.

The respiration rates per unit leaf area (R_{sla}) obtained during Experiment III in May 2008 (Table 2.4) were significantly higher than those measured in July at similar temperatures (18 ºC) during day 3 of experiment IV (0.57 µmol CO_2 m^{-2} s^{-1}, data not shown). Besides possible phenological effects, this discrepancy is already explained for the most part by the difference in LMA between the two individual trees - other than that very similar-: 234 to 275 g m^{-2} in Experiment III (Table 2.4) *vs.* 182 g m^{-2} in Experiment IV.

Regular measurements of R_{plant} like those made in Experiment 1, including growth and maintenance components, are of limited help for linking CO_2 efflux to specific physiological processes - and thus for modelling orchards carbon balance. Maintenance respiration is theoretically explained in its most part by the environment temperature; it is therefore among the two components the easiest to model. A simple exercise to estimate plant R_m in each measurement of Experiment I is to apply the mass-specific R_m rates for leaf, fruits, fine branches (from Experiment II) and for wood (from Experiment IV) to the respective amount of plant components (estimated) in Experiment I. The difference between the estimated R_m and the measured R_{plant} in each period may be attributed to growth respiration, which resulted to be 36% (October 2006), 31% (December 2006), 49% (October 2007), 73% (May 2008) and 33% (July 2008) of the total R_{plant} (Fig. 5.4).

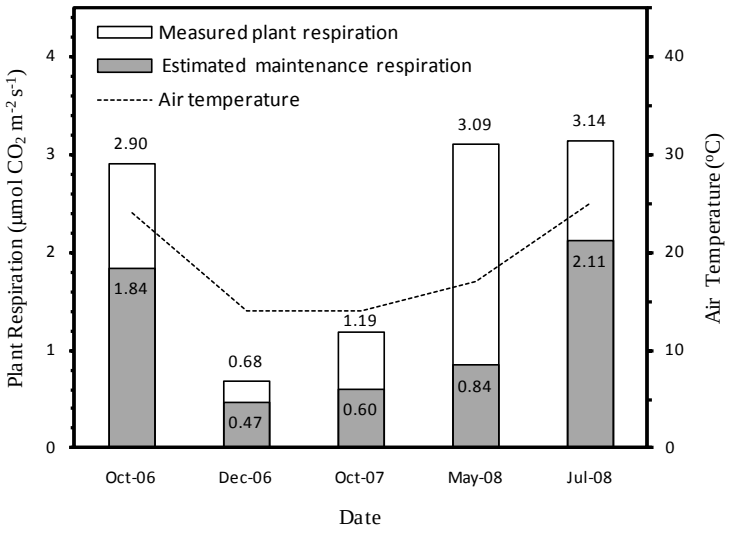

Figure 5.4. Plant aboveground respiration measured in October 2006, December 2006, October 2007, May 2008 and June 2008, Experiment I. Maintenance respiration was estimated using the relationships with temperature for different organs measured in Experiment II and for wood in Experiment IV.

With the exception of October 2007 and May 2008, the percentage of growth to total respiration was close to that found in experiment II with prolonged darkness (30%). The largest discrepancy occurred during flowering (in May 2008) when aboveground growth is at its peak. This is consistent with the findings of Rambal *et al.* (2004): they estimated growth respiration as the residual between measured R_{eco} and base R_{eco} in a *Quercus ilex* Mediterranean forest, finding similar high growth respiration during flowering (May also in that case). The value of growth respiration for October is also quite high: The effect of growth respiration over the variability of R_{plant} is quite important: no model of carbon balance for olive orchards will be reliable over the whole season unless growth respiration is explicitly taken into account.

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References

Angelopoulos K, Dichio B, Xiloyannis lC. 1996. Inhibition of photosynthesis in olive trees (Olea europaea L.) during water stress and rewatering. Journal of Experimental Botany, 47(301): 1093-1100.

Atkin OK, Holly C, Ball MC. 2000. Acclimation of snow gum (*Eucalyptus pauciflora*) leaf respiration to seasonal and diurnal variations in temperature: the importance of changes in the capacity and temperature sensitivity of respiration. Plant, Cell and Environment, 23(1): 15-26.

Atkin OK, Scheurwater I, Pons T. 2006. High thermal acclimation potential of both photosynthesis and respiration in two lowland Plantago species in contrast to an alpine congeneric. Global Change Biology, 12(3): 500-515.

Baldocchi D. 2008. Turner Review No. 15. 'Breathing' of the terrestrial biosphere: Lessons learned from a global network of carbon dioxide flux measurement systems. Australian Journal of Botany, 56(1): 1-26.

Bunce JA. 2007. Direct and acclimatory responses of dark respiration and translocation to temperature. Annals of Botany, 100(1): 67-73.

Cannell MGR, Thornley JHM. 2000. Modelling the Components of Plant Respiration: Some Guiding Principles. Annals of Botany, 85(1): 45-54.

Damesin C, Ceschia E, Goff NL, Ottorini JM, Dufrêne E. 2002. Stem and Branch Respiration of Beech: From Tree Measurements to Estimations at the Stand Level. New Phytologist, 153(1): 159-172.

Diaz-Espejo A, Walcroft AS, Fernandez JE, Hafidi B, Palomo MJ, Giron IF. 2006. Modeling photosynthesis in olive leaves under drought conditions. Tree Physiology, 26(11): 1445-1456.

Falge E, Baldocchi D, Tenhunen J, Aubinet M, Bakwin P, Berbigier P, Bernhofer C, Burba G, Clement R, Davis KJ et al. 2002. Seasonality of ecosystem respiration and gross primary production as derived from FLUXNET measurements. Agricultural and Forest Meteorology, 113(1-4): 53-74.

Gifford RM. 2003. Plant respiration in productivity models: Conceptualisation, representation and issues for global terrestrial carbon-cycle research. Functional Plant Biology, 30(2): 171-186.

Hanson PJ, Edwards NT, Garten CT, Andrews JA. 2000. Separating root and soil microbial contributions to soil respiration: A review of methods and observations. *Biogeochemistry* 48(1): 115-146.

Law BE, Falge E, Gu L, Baldocchi DD, Bakwin P, Berbigier P, Davis K, Dolman AJ, Falk M, Fuentes JD et al. 2002. Environmental controls over carbon dioxide and water vapor exchange of terrestrial vegetation. Agricultural and Forest Meteorology, 113(1-4): 97-120.

Lloyd J, Taylor JA. 1994. On the Temperature Dependence of Soil Respiration. Functional Ecology, 8(3): 315-323.

McCree KJ, Silsbury JH. 1978. Growth and Maintenance Requirements of Subterranean Clover. Crop Science, 18(1): 13-18.

McCree KJ, Amthor ME. 1982. Effects of diurnal variation in temperature on the carbon balances of white clover plants. Crop Science, 22: 822-827.

Moriana A, Villalobos FJ, Fereres E. 2002. Stomatal and photosynthetic responses of olive (*Olea europaea L.*) leaves to water deficits. Plant, Cell and Environment, 25(3): 395-405.

Owen KE, Tenhunen J, Reichstein M, Wang Q, Falge E, Geyer R, Xiao X, Stoy P, Ammann C, Arain A et al. 2007. Linking flux network measurements to continental scale simulations: Ecosystem carbon dioxide exchange capacity under non-water-stressed conditions. Global Change Biology, 13(4): 734-760.

Perez-Priego O, Testi L, Orgaz F, Villalobos FJ. 2010. A large Closed Canopy-Chamber for measuring CO_2 and water vapour exchange of whole trees. Environmental and Experimental Botany, 68(2): 131-138.

Proietti P, Famiani F, Tombesi A. 1999. Gas exchange in olive fruit. Photosynthetica, 36(3): 423-432.

Rambal S, Joffre R, Ourcival JM, Cavender-Bares J, Rocheteau A. 2004. The growth respiration component in eddy CO2 flux from a Quercus ilex mediterranean forest. Global Change Biology, 10(9): 1460-1469.

Reichstein M, Tenhunen J, Roupsard O, Ourcival JM, Rambal S, Miglietta F, Peressotti A, Pecchiari M, Tirone G, Valentini R. 2003. Inverse modeling of seasonal drought effects on canopy CO_2/H_2O exchange in three Mediterranean ecosystems. Journal of Geophysical Research D: Atmospheres, 108(23).

Ryan M, Linder S, Vose J, Hubbard R. 1994. Dark respiration of pines. Ecological Bulletins, 43: 50-63.

Ryan MG, Hubbard RM, Pongracic S, Raison RJ, McMurtrie RE. 1996. Foliage, fine-root, woody-tissue and stand respiration in Pinus radiata in relation to nitrogen status. Tree Physiology, 16(3): 333-343.

Ryan MG, Lavigne MB, Gower ST. 1997. Annual carbon cost of autotrophic respiration in boreal forest ecosystems in relation to species and climate. Journal of Geophysical Research D: Atmospheres, 102(24): 28871-28883.

Smith P, Lanigan G, Kutsch WL, Buchmann N, Eugster W, Aubinet M, Ceschia E, Béziat P, Yeluripati JB, Osborne B et al. 2010. Measurements necessary for assessing the net ecosystem carbon budget of croplands. Agriculture, Ecosystems and Environment doi: DOI: 10.1016/j.agee.2010.04.004.

Testi L, Orgaz F, Villalobos F. 2008. Carbon exchange and water use efficiency of a growing, irrigated olive orchard. Environmental and Experimental Botany, 63(1-3): 168-177.

Thornley JHM, Cannell MGR. 2000. Modelling the components of plant respiration: Representation and realism. Annals of Botany, 85(1): 55-67.

Valentini R, Matteucci G, Dolman AJ, Schulze ED, Rebmann C, Moors EJ, Granier A, Gross P, Jensen NO, Pilegaard K et al. 2000. Respiration as the main determinant of carbon balance in European forests. Nature, 404(6780): 861-865.

Villalobos FJ, Testi L, Hidalgo J, Pastor M, Orgaz F. 2006. Modelling potential growth and yield of olive (Olea europaea L.) canopies. European Journal of Agronomy, 24(4): 296-303.

General discussion and final remarks

1. General discussion

The capacity of olive canopies for carbon fixation in different environments is almost unknown despite that their importance in Mediterranean agricultural systems. Besides this ecological function, most irrigated olive orchards have not enough water to meet the maximum crop water requirements. In this sense, irrigated agriculture under a water scarcity scenario demands the development of deficit irrigation strategies for maximizing biomass accumulation and yield. To gain insight about the yield response of olive to water deficit irrigation, the carbon assimilation, transpiration and water productivity of olive trees have to be investigated. The main objective of this thesis is to characterize the CO_2 exchange of olive orchard under different environmental conditions and its relation with water use. Nevertheless, the first question is "How direct flux measurements of CO_2 and water vapour can be performed at tree scale in an olive for different levels of water deficit?"

At the orchard scale, the measurement of CO_2 and water vapour gas exchange is usually performed using micrometeorological techniques. Eddy covariance measurement of CO_2 and water vapour exchanges has been commonly used in recent years (e.g. Euroflux and Ameriflux projects, Falge et al. 2002). This technique relies on strong assumptions for flux corrections, is difficult to apply and requires large, uniform and flat plots. Eddy covariance measures a net flux and different processes contributing to the measured net ecosystem exchange cannot be distinguished using only this technique. Therefore, many other supplementary measurements are needed for the assessment of the carbon budget (Smith et al., 2010). A suitable method for measuring the gas exchange of individual trees is the use of large canopy chambers. Nevertheless, there

are practical limitations in the design, construction and operation of chambers large enough to enclose a whole tree.

A goal of this work is the development of a technique to measure canopy gas exchange of single trees in the field. Chapter 1 includes the description and testing of a large closed chamber of the transient-state type for measuring the CO_2 and water vapour exchange rates of tree crowns with volumes up to 20-30 m^3. Transient-state chambers have the main advantage of a minimal disturbance of the environment around the tree as compared with steady-state chambers but they require to be operated manually on site limiting data collection, mainly during the night.

The chamber presented operates as a transitory-closed system (Steduto et al., 2002) and consists of a hexagonal prism with basal area of 10.4 m^2 and height of 4 m. The windows and roof, which are open and closed manually, are made of a transparent thick polyester film, stretched and fixed to frames made of aluminium. The base of the chamber is sealed around the tree trunk through a 2-mm thick polyethylene sheet. The measuring protocol is as follows: Just before measuring, the windows and top have to be closed and four 80-W fans are turned on. A vacuum pump takes the air from intake points distributed through the chamber and then returns it to the chamber. A sample of 1 L min^{-1} of this air flow is sniffed using a small pump and diverted to a CO_2/H_2O infrared gas analyser (IRGA) which measures gases concentrations at 1 Hz sampling rate; the output is recorded by a datalogger. After each measurement, the fans are turned off and the chamber goes back to the open position. The rate of change of the concentration of CO_2 and H_2O is the apparent flux of net photosynthesis and transpiration, respectively. The calculations take into account the correction for air temperature and atmospheric pressure inside the chamber (Reicosky et al. 1990) and use the quadratic regression model proposed by Wagner et al. (1997) in order to reduce the error due to non linear gas concentration change inside closed systems.

The tests carried out showed that the large chamber volume reduced the impact of the main sources of error in calculating the gas exchange flux in this type of chambers, namely: leakage, adsorption, canopy and air heating, and leaf/air gradient reduction. However, it is not easy to predict *a priori* the effect of a ventilated chamber over the gas exchange rate of an enclosed plant. The changes in the concentration of the confined atmosphere gases may have complex effects on many physiological processes. The results of the sap velocity experiment proved that the effect of long exposition to the enclosed environment reduced the transpiration rate, and the reduction appeared to be directly proportional to the closure duration. Consequently, the chamber should be closed during the shortest time possible to avoid underestimation of water vapour, and - presumably - CO_2 fluxes. Under normal measurement conditions, with closure durations between 100 and 180 s, the transpiration at the time of re-opening were reduced by less than 5%. It should be emphasised that this underestimation is only a potential disturbance; it will not affect the gas exchange measurements when the calculations are made with the quadratic method (where the rate is taken from the slope at the beginning of the calculation window).

Canopy conductance (G_c) of olive trees was barely affected by chamber closure, and only after a closure duration that exceeds by much the typical measurement durations. Therefore, the transpiration reduction was mainly the result of changes in the vapour pressure gradient. Unfortunately, we had no independent measurements of CO_2 assimilation (as we had of transpiration) to explicitly prove that the overall change in the rate of CO_2 flux due to canopy enclosing is irrelevant. Nevertheless, the relative changes in the leaf/chamber CO_2 gradient occurring during a measurement are by far smaller than the ones in water vapour concentration, thus, as G_c is almost constant, the changes in assimilation (or respiration) rates induced by the chamber must be very small. The minimal effects that the chamber generated on the physiological processes of the enclosed tree indicate that this chamber design is suitable for accurate measurements of gas exchange at tree level in almost all the cultivated fruit trees.

In Chapter 2, diurnal and seasonal variations of canopy assimilation (A_n), transpiration (E) and canopy conductance (G_c) of olive trees were characterized during a two-year study in an experiment where regulated deficit irrigation (RDI) was compared with a well irrigated control (CI). Additionally, the evaluation of the effect of water stress on water use efficiency was studied. These data are the first tree-level measurements of carbon and water exchange in large olive trees. Besides, we used simultaneous measurements of soil respiration for up-scaling instantaneous carbon fluxes from canopies to orchard levels and thus net ecosystem fluxes (NEE) were estimated. For the sake of comparison, these estimations were consistent with those reported by Testi et al. (2008) with eddy covariance technique. Furthermore, we estimated a Radiation-Use efficiency (RUE) value of 0.9 g dry matter (MJ PAR)$^{-1}$ for the control tree which is close to the values found by Mariscal et al. (2000) and Villalobos et al. (2006). A similar analysis led to slightly higher RUE for the RDI tree (1.01 g dry matter (MJ PAR)$^{-1}$.

The water use efficiency (WUE) is a key parameter in crop simulation models that evaluate optimum water use by analyzing the response of plants to water stress. WUE is not only essential to improve the irrigation management but also to quantify biomass, water use, productivity and carbon exchange of olive ecosystems under different climatic and management scenarios. The biomass produced under well-watered conditions can be estimated with a simple function of the relatively stable WUE and the easy-to-obtain vapour pressure deficit (VPD). However, if WUE is unresponsive to water stress, or if its response can be predicted, WUE models could be still valid or easily adaptable to water stress conditions; unfortunately these assumptions are not yet confirmed. Therefore, this work aimed to answer the question of whether moderate water stress (by deficit irrigation) increases the WUE or not.

The results presented in Chapter 2 showed that water stress had a small effect on instantaneous WUE during the central hours of the day with differences typically below

10%, which is in contrast with Moriana et al. (2002) who did not find differences in instantaneous leaf WUE among different irrigation treatments in olive trees even under moderate water stress. However, mean diurnal WUE (WUE_m) increased strongly under water stress reaching up to 78% increase (Table 3.3). This effect is explained by an increase in WUE during the morning, when VPD was lower and by the relative variation in stomatal opening in the RDI tree which reduces the contribution of high VPD to the daily transpiration as compared with the control. Mathematically WUE_m is the transpiration-weighted average of WUE. Therefore for a decreasing diurnal pattern of WUE (Fig. 4.3), a decrease of transpiration in the afternoon (when WUE is low) leads to higher WUE_m as the contribution of the early morning (high WUE) is enhanced. The same analysis would apply when considering longer (seasonal) time scales when it is possible to impose a change in the relative distribution of transpiration by irrigation management. Reducing applied irrigation during summer would shift the relative transpiration distribution to conditions of higher WUE, thus improving seasonal WUE. Important implications are derived from this behaviour. For instance, crop models of daily time step based on WUE for biomass accumulation (e.g. Cropsyst, Stöckle et al., 2003) would underestimate biomass production of water stressed crops if the relationship between WUE and VPD is calibrated using well irrigated crops.

The measurements allowed the calibration of a conductance model coupling canopy conductance and assimilation. The conductance model was used for understanding variations in WUE associated with the different water status. Equation 4 (Chapter 2) shows that WUE will decrease when any of the parameters of the Ball-Berry-Leuning model (G_0, b and D_0) increases or when stomata close (decrease in G_c) (Leuning, 1995; Dewar, 1995) . According to Dewar (2002) the product of parameters b and D_0 is proportional to K, the hydraulic conductance between the bulk leaf and the guard cells, which decreases with leaf water potential. In physiological terms water deficit (low leaf water potential) reduces the hydraulic connection between the bulk leaf and the guard cells thus enhancing stomatal closure in response to high VPD. Therefore, the increase

in WUE with moderate water deficit found in this experiment may be explained by the reduction of the product $b \cdot D_0$.

The overall improvement of WUE in olive trees under water deficit supports the use of deficit irrigation strategies for maximizing biomass accumulation when water stress is concentrated in the summer (high VPD). Further research should define the limits of water deficits to avoid the negative side effects (reduced harvest index, leaf senescence, etc) that could neglect the benefits mentioned above.

The capacity of olive orchards for carbon sequestration is regulated by the balance between photosynthesis (C gain) and respiration (C release). For reliable estimations at the stand level, information about the magnitude of crown net assimilation and respiration in photosynthetic and non-photosynthetic plant organs is required. However, those measurements are difficult to perform in the field and scaling procedures are not always straightforward. Chapter 3 presents the patterns of plant (R_{plant}) and soil respiration (R_{soil}) in olive orchards and includes four experiments offering new insights on the response of respiration of different organs.

In Experiment I we obtained quasi-simultaneous measurements of plant (R_{plant}) and soil respiration (R_{soil}**)** during 3 years. R_{soil} showed little or no dependence on the seasonal temperature cycle. Although R_{soil} was always higher in the wet zone than in non-wetted soil of the alleys, it did not increase after the rain events in the autumn with still warm soil temperatures. This time-spatial pattern of soil respiration may be explained by the interaction of soil water content and root distribution as high root density and microbial activity overlap in the wet zones only. Fig. 1.4 shows that R_{soil} was higher than R_{plant} when temperature was lower than 20 ºC; Total R_{soil} was almost constant, whereas R_{plant} varied proportionally with air temperature.

We observed a strong variability in the mass-specific R_{plant} at a common measuring temperature (around 20ºC) among data obtained in October 2006, May 2008 and July

2008. Part of this variability may be due to the different contribution of the growth component of R_{plant} when the tree allocates most of the new assimilates to different organs in different seasons: shoots and flowers during the measurements taken in May and fruits in July and October.

Although there is not a rigorous division between growth and maintenance energy-requiring processes (Cannell & Thornley, 2000). Experiment II showed that the respiration rates after 6-8 h of darkness were higher than those after 48 h and directly proportional to temperature. Upon the assumption that the latter are representative of maintenance metabolism, our results indicate that respiration in young olive trees could be divided roughly in 30% for growth and 70% for maintenance.

The results of experiment III (Table 2.4) over three nights in May 2008 (at almost constant temperature) confirms that total respiration rate is tightly related to dry mass composition and leaf area. Mass-specific plant respiration (R_{spm}) decreased significantly as the non-woody fraction was reduced. Respiration on a leaf mass basis (R_{slm}) showed no significant differences among the 3 days.

Experiment IV confirmed that the trunk and branches have much lower respiration rates than non-woody organs (an expected result, as wood contains mostly structural material with very little or null metabolic requirements). Respiration of the trunk accounted for 6 % of the total above-ground R_{plant} at 18 ºC. The small maintenance cost of structural wood indicates that aboveground R_{plant} is weakly affected by differences in woody biomass, while leaves are the main contributors.

Regular measurements of R_{plant} like those made in Experiment 1, including growth and maintenance components, are of limited help for linking CO_2 efflux to specific physiological processes - and thus for modelling orchards carbon balance. Maintenance respiration is mostly explained by temperature, and thus, it is the easiest to model of the two components. A simple exercise to estimate plant R_m in each measurement of

Experiment I was performed by applying the mass-specific R_m rates for leaf, fruits, fine branches (from Experiment II) and for wood (from Experiment IV) to the estimated amount of plant components in Experiment I at a reference temperature. The difference between the estimated R_m and the measured R_{plant} in each period was attributed to growth respiration. We observed that the ratio of growth to total respiration was close to that found in experiment II with prolonged darkness (30%). However, the largest discrepancy occurred during flowering (in May 2008) when aboveground growth is at its peak. The effect of growth respiration over the variability of R_{plant} is quite important: no model of carbon balance for olive orchards will be reliable over the whole season unless growth respiration is explicitly taken into account.

2. Final Remarks

1. This work demonstrates that it is possible to measure the CO_2 and water vapour exchange rates of trees with volumes up to 20-30 m^3 by a large chamber of the transient-state type.

2. Field tests showed that this chamber imposes minimum disturbance on the canopy environment, and that no appreciable alteration in canopy conductance is generated during standard measurements. The minimal effects that the chamber generated over the physiological processes of the enclosed tree indicate that this chamber design is suitable for accurate measures of gas exchange at tree level in almost all the cultivated fruit tree species.

3. Diurnal and seasonal variations of canopy assimilation (A_n), transpiration (E) and canopy conductance (G_c) of olive trees were characterized during a two-year study in an experiment where deficit irrigation strategies were compared with a well irrigated control, being A_n higher for CI (9.6-22.0 g CO_2 m^{-2} day^{-1}) than for RDI (8.0-19.4 g CO_2 m^{-2} day^{-1}).

4. Instantaneous Water Use Efficiency measured in CI and RDI showed a decreasing diurnal pattern and were inversely related to VPD.

5. The measurements allowed the calibration of a model coupling canopy conductance and assimilation that contributed to evaluate the effect of water stress on water use efficiency.

6. While water stress improved instantaneous WUE only slightly, the effect was dramatic for daily values. This effect is explained by an increase in WUE during the morning, when VPD was lower and by the relative variation in stomatal opening in the RDI tree which reduces the contribution of high VPD to the daily transpiration as compared with the control.

7. Extrapolating this analysis to longer (seasonal) time scales when it is possible to impose a change in the relative distribution of transpiration by irrigation management by reducing applied irrigation during summer would shift the relative transpiration distribution to conditions of higher WUE, thus improving seasonal WUE.

8. The overall improvement of WUE in olive trees under water deficit supports the use of deficit irrigation strategies for maximizing biomass accumulation when water stress is concentrated in the summer (high VPD). Further research should define the limits of water deficits to avoid the negative side effects.

9. Soil respiration was a large component of ecosystem respiration and was strongly limited in dry soil. Nevertheless, respiratory activities of plant roots, of the mycorrhizal fungi and decomposers organism (heterotrophs) in soils remain uncertain and require further studies.

10. While soil respiration was almost constant, plant respiration varied in proportion to temperature. Soil respiration was the largest contributor to ecosystem respiration when air temperature was lower than 20ºC.

11. Approximately 30% of plant respiration was associated to growth and 70% to maintenance respiration, whose dependence with temperature was empirically determined for different organs. The maintenance coefficient in fruits was closed to that found in leaves, while the coefficient for branches was the lowest.

12. Plant respiration was more closely related to leaf mass than plant mass or leaf area. Therefore, leaves were the main contributors to aboveground plant respiration.

13. Respiration of the trunk accounted for 6 % of the total above-ground plant respiration of a large tree at 18 ºC. The small maintenance cost of structural wood indicates that above-ground plant respiration is weakly affected by differences in woody biomass.

References

Cannell MGR, Thornley JHM. 2000. Modelling the components of plant respiration: some guiding principles. Annals of Botany, 85(1): 45-54.

Dewar RC. 1995. Interpretation of an empirical model for stomatal conductance in terms of guard cell function. Plant, Cell and Environment, 18: 365-372.

Dewar RC. 2002. The Ball-Berry-Leuning and Tardieu-Davies stomatal models: synthesis and extension within a spatially aggregated picture of guard cell function. Plant, Cell and Environment, 25: 1383-1398.

Falge E, Baldocchi D, Tenhunen J, Aubinet M, Bakwin P, Berbigier P, Bernhofer C, Burba G, Clement R, Davis KJ, Elbers JA, Goldstein AH, Grelle A, Granier A, Gumundsson J, Hollinger D, Kowalski AS, Katul G, Law BE, Malhi Y, Meyers T, Monson RK, Munger JW, Oechel W, Paw U KT, Pilegaard K, Rannik U, Rebmann C, Suyker A, Valentini R, Wilson K, Wofsy S. 2002. Seasonality of ecosystem respiration and gross primary production as derived from FLUXNET measurements. Agricultural and Forest Meteorology, 113(1-4): 53-74.

Leuning R. 1995. A critical appraisal of a combined stomatal-photosynthesis model for C3 plants. Plant, Cell and Environment, 18(4): 339-355.

Mariscal MJ, Orgaz F, Villalobos FJ. 2000. Radiation-use efficiency and dry matter partitioning of a young olive (Olea europaea) orchard. Tree Physiology, 20(1): 65-72.

Moriana A, Villalobos FJ, Fereres E. 2002. Stomatal and photosynthetic responses of olive (Olea europaea L.) leaves to water deficits. Plant Cell and Environment, 25(3): 395-405.

Reicosky DC. 1990. A portable chamber for evapotranspiration measurements and irrigation scheduling. Acta Horticulturae 278, 455–461.

Smith P, Lanigan G, Kutsch WL, Buchmann N, Eugster W, Aubinet M, Ceschia E, Béziat P, Yeluripati JB, Osborne B, Moors EJ, Brut A, Wattenbach M, Saunders M, Jones M. 2010. Measurements necessary for assessing the net ecosystem carbon budget of croplands. Agriculture, Ecosystems and Environment, doi: DOI: 10.1016/j.agee.2010.04.004.

Stöckle CO, Donatelli M, Nelson R. 2003. CropSyst, a cropping systems simulation model. European Journal of Agronomy, 18(3-4): 289-307.

Steduto P, Çetinkökü Ö, Albrizio R, Kanber R. 2002. Automated closed-system canopy-chamber for continuous field-crop monitoring of CO2 and H2O fluxes. Agricultural and Forest Meteorology, 111(3): 171-186.

Testi L, Orgaz F, Villalobos F. 2008. Carbon exchange and water use efficiency of a growing, irrigated olive orchard. Environmental and Experimental Botany, 63(1-3): 168-177.

Villalobos FJ, Testi L, Hidalgo J, Pastor M, Orgaz F. 2006. Modelling potential growth and yield of olive (Olea europaea L.) canopies. European Journal of Agronomy, 24(4): 296-303.

Wagner SW, Reicosky DC, Saamuel Alessi R. 1997. Regression models for calculating gas fluxes measured with a closed chamber. Agronomy Journal 89, 279–284.

Additional material

Fig. 1. Outlook of the camber during the construction phase in the laboratories of the Instituto de Agricultura Sostenible (IAS).

Fig 2. Chamber ready to be tested outside of the laboratories of the Instituto de Agricultura Sostenible (IAS).

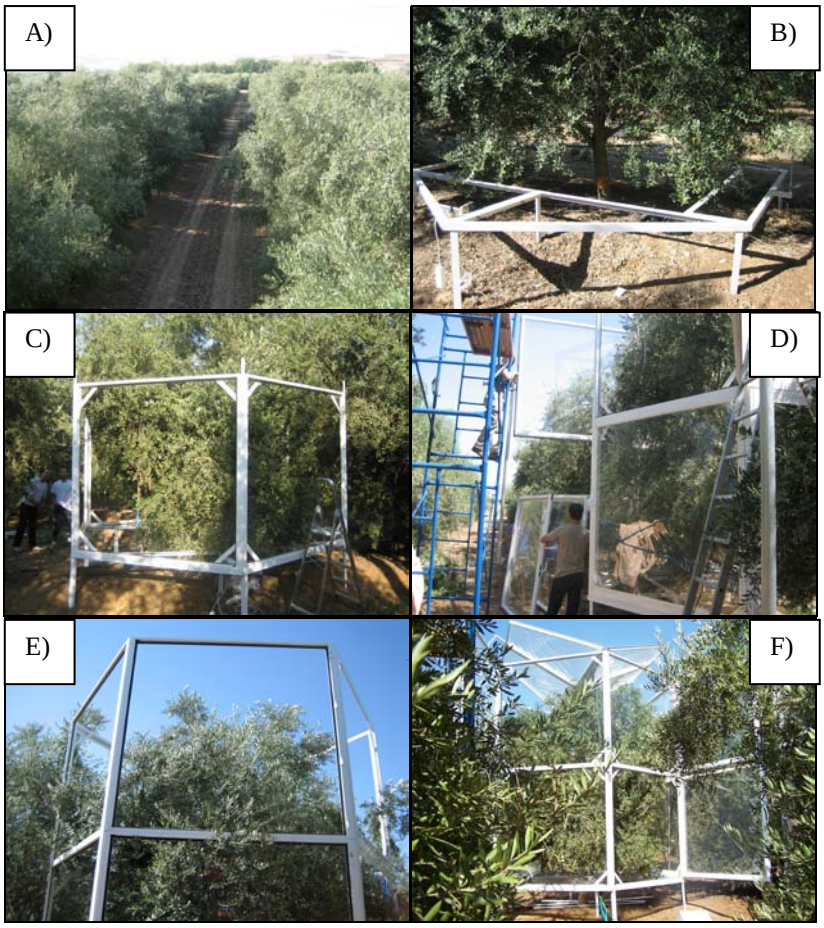

Fig 3. A) Outlook of the olive orchard where the chamber was placed. B) Shows the firm stainless steel frame with sharp supports sunk in the soil where the whole chamber is mounted around the tree. C), D), E) and F) Show how the chamber structure is being assembled with 12 sections of rigid aluminium frames (2 m x 2 m) that are easy fitted and screwed together. Finally, the top and four of the six walls are hinged windows, which allow quickly opening and closing the chamber.

Fig. 4. The chamber is normally kept with all the windows and top in the open position as is shown in these pictures causing minimum disturbance to the tree environment.

Fig 5.Before measuring, the windows and top are closed and fans turned on. The CO_2 and water vapour concentrations, measured by the IRGA, change steadily after a short lag time. The rate of change of both gases with time is the apparent flux of assimilation or respiration and transpiration, respectively, of the tree. After the measurement is completed, the chamber goes back to the open position as Fig. 4, with the fans off until the following measurement.

Fig. 6. A measurement of plant respiration during the night.

Curriculum Vitae of the author

Oscar Pérez Priego was born on 29th of August 1976 in Ecija (Sevilla). He received the degree in forest engineering from the University of Córdoba, Spain in 2004. After that he performed his Mater thesis in remote sensing at the Institute for Sustainable Agriculture, Spanish Council for Scientific Research, Córdoba, Spain. His work concerned on detection of water stress through chlorophyll fluorescence, and methods based on high-resolution spectrometry at leaf and canopy levels for biochemistry and biophysical estimates using reflectance spectra.

In 2005 he started the PhD program of "Biosciences" at Agronomy Department of University of Cordoba with a ministry of science and innovation fellowship funded through the FPI Program. To present his research interest is aimed to understand interactions and dynamics of carbon and water resources in olive ecosystems. He is particularly interested in how ecophysiological processes are manifested at plant level. He is currently working on carbon balance of olive orchards in relation with water supply. He has measured with large closed tree chambers comparing the water and C exchange of irrigated and water-limited trees. He has also used small soil respiration chambers.

During this period he performed two research stays abroad at: Centre for Land Rehabilitation of the School of Earth and Geographical Sciences, Faculty of Natural and Agricultural Sciences, University of Western Australia (January-April 2007, Perth, Australia) where he was engaged in collaborative research with Dr Mark Tibbett with the purpose of studying the effect of elevated atmospheric CO_2 concentration in free-air CO_2 enrichment (FACE) plots on soil carbon dynamic; and the Laboratory of Forest Ecology of the Department of Forest Science and Environment University of Tuscia (January-April 2008, Viterbo, Italia), with the supervision of the Prof. Riccardo Valentini focused on quantify a temporal carbon balance in canola, and how it was affected by different management practices.

Peer reviewed publications in international journals

1. Villalobos FJ, **Pérez-Priego O**, Testi L, Orgaz F. Effects of water supply on carbon and water exchange of olive trees. European Journal of Agronomy (submitted).
2. **Pérez-Priego O**, Testi L, Orgaz F,Villalobos FJ. Soil and plant respiration of an olive orchard.(In preparation).
3. Rapoport HF, Hammami BM, Martins P, **Pérez -Priego O**, Orgaz F. Influence of water deficits at different times during olive tree inflorescence and flower development. Environmental and Experimental Botany (submitted).
4. **Pérez-Priego O**, Testi L, Orgaz F, Villalobos FJ. 2010. A large Closed Canopy-Chamber for measuring CO_2 and water vapour exchange of whole trees. Environmental and Experimental Botany. 68: 131-138.
5. Suárez L, Zarco-Tejada PJ, Sepulcre-Cantó G, **Pérez-Priego O**, Miller JR, Jiménez-Muñoz JC, Sobrino J. 2008. Assessing canopy PRI for water stress detection with diurnal airborne imagery. Remote Sensing of Environment. 112: 560-575.
6. **Oscar Perez-Priego**, Pablo J. Zarco-Tejada, John R. Miller and Elías Fereres. 2005. Detection of water Stress in Orchard trees With a High-Resolution Spectrometer Through Chlorophyll Fluorescence In-Filling of the O2-A Band. IEEE Transactions on Geoscience and Remote Sensing. 43: 2860- 2869.

Proceedings, Symposia, courses and Conferences

- 24th New Phytologist Symposium of Plant respiration and climate change: scaling from mitochondria to the globe.). St Hugh's College, University of Oxford, UK. 11–14 April 2010.
- Adaptive Management of Mediterranean Forest Ecosystems to Climate Change. Course in advance.Instituto Agronómico Mediterraneo de Zaragoza

(IAMZ). Centro Internacional de Altos Estudios Agronómicos Mediterráneos (CIHEAM). Zaragoza, España. 10-15 May 2010.

- International Symposium on Olive Irrigation and Oil Quality. Agricultural Research Organization of Israel. Poster: Water deficit effect during olive tree inflorescence and flower development. Co-author: Hava F. Rapoport, Paula Martins, **Oscar Pérez-Priego**, Francisco Orgaz. Nazareth, Israel. 6-10 December 2009.
- Forest and water stress in a changing environment: from cell to ecosystems. Course in advance. COST Dynamic Training School. Supported by: COST FP 0601 (FORMAN - Forest Management and the Water Cycle) and COST FA 0603 (PPE - Plant Proteomics in Europe). Orvieto, Italia. 20-23 May 2009.
- X Congress of the European Society for Agronomy. Bologna, Italy. 15-19 September 2008. **Pérez-Priego O.**,Testi L., Orgaz F.,Villalobos F.J. Measuring the carbon balance of olive trees using chambers. 2008. Italian Journal of Agronomy. 3: 865-866.
- Carbon and Greenhouse Gases in agricultural and forest ecosystems: Mitigation options and the Kyoto protocol. Course in advance. University of Barcelona. Bellaterra-Barcelona, Spain. 27 March-1 April 2006.
- Simulations with FluorMOD. Airborne Imaging Spectroscopy Workshop. Poster: Zarco-Tejada, P.J., G. Sepulcre-Cantó, O. **Pérez-Priego**, J.A. Sobrino, J.C. Jiménez-Muñoz, A. Berjón, P. Martín, R. González, Water Stress and Fluorescence Detection in Crop Canopies with AHS Thermal Imagery. Bruges, Belgium. 7 October 2005.
- 2nd International Workshop on Remote Sensing of Vegetation Fluorescence. Poster: Zarco-Tejada, P.J., **O. Pérez-Priego**, G. Sepulcre-Cantó, J.R. Miller, E. Fereres, Chlorophyll Fluorescence Detection with a High-Spectral Resolution Spectrometer through in-filling of the O2-A band as function of Water Stress in Olive Trees. Montreal, Canada. 17-19 November 2004.