Research paper

Impact of root growth and hydraulic conductance on canopy carbon-water relations of young walnut trees (*Juglans regia* L.) under drought

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ABSTRACT

In addition to soil characteristics, two plant traits control the supply of water from the soil to the canopy: root growth and plant hydraulic conductance. Here we examine the impact of root growth and hydraulic conductance on water uptake and transpiration of walnut under deficit irrigation. A greenhouse experiment was conducted using nine young walnut trees (*Juglans regia* L.) grown for three months in transparent pots, equipped with: (i) rhizotron tubes, which allowed for non-invasive monitoring of root growth; (ii) pressure transducer tensiometers, recording soil water potential at soil-root interfaces; (iii) psychrometers attached to mature leaves, measuring stem water potential; and (iv) weighing scales used to determine total plant transpiration. Irrigation treatments consisted of different replenishment levels (100%, 75%, and 50%) of potential transpiration replicated over time. Walnut trees showed rapid physiological acclimation characterized by a fast decline and subsequent stabilization of transpiration rates soon after the beginning of drought stress treatments. We also observed a significant decrease in plant hydraulic conductance with decreasing soil and stem water potential under drought stress. At the end of the experiment, isotopic measurements revealed the integrated effect of physiological acclimation on canopy carbon-water relations. Leaf carbon isotope ratios showed significant increases in water-use efficiency with deficit irrigation levels. Leaf water hydrogen and oxygen isotope ratios confirmed that changes in water use-efficiency were caused by decreases in transpiration. Conversely, root growth was highest under low stress (T100) and lowest under high stress (T50). These results indicate the existence of a fundamental tradeoff between water-use efficiency and root growth, which will be useful to optimize the application of water and improve the design of irrigation systems in walnut orchards.

1. Introduction

Walnut (*Juglans regia* L.) is a tree species of great economic importance, particularly in the Central Valley of California (DANR/UC, 2014), which provides 99% of the US commercial supply and 66% of the worldwide production of walnut kernels (California Walnut Board, 2015). In California, the majority of walnut orchards are located in areas that are periodically affected by drought. In recent years, drought stress has led to increased tree mortality and a decline in walnut productivity across the state (USDA, 2014). Identifying how plant traits control the supply of water from the soil to the canopy is of high relevance in order to optimize water application while maintaining orchard productivity under increasing climatic variability.

Walnut trees have high water requirements. Their growth is strongly affected by water deficit, which results in decreased yield, deep bark canker, and low kernel size and quality, among other issues (Lampinen et al., 2003; Buchner et al., 2008). In contrast, early seasonal over-irrigation can cause *Phytophthora* root rot and dieback (Lampinen et al., 2003). In addition, both nitrate deficit (e.g. after its leaching due to excessive irrigation) and climate seasonality can alter root-to-shoot growth allocation (Silva et al., 2015a; Sperling et al., 2017) jeopardizing the sustainability of tree growing operations (Prichard, 1998;

Di and Cameron, 2002). As in other parts of the world currently experiencing changes in climate, the increasingly frequent drought events in California call for adjusted water management, which requires understanding of the relationship between water application and tree transpiration to avoid the undesirable effects of limited and excessive irrigation.

The soil water that is available for plants is held by soil matric forces between field capacity (i.e. −0.01 MPa for coarse textured soils and −0.03 MPa for fine textured soils) and the permanent plant wilting point (i.e. from −1.5 MPa up to −6 MPa, depending on plant species and its capacity to take up water from soil) (Salisbury and Ross, 1978; Veihmeyer and Hendrickson, 1927). This notion has been revised due to the fact that only a fraction of the total available water in the root zone is "readily" available (Allen et al., 1998), while another fraction of soil water is available at longer-term. In other words, from a hydrological perspective, plant water availability is "rate limited" by hydraulic impedances on the pathways of water (Couvreur et al., 2014b). Three main properties are thought to control the flow rate-limitation. The first one is the soil hydraulic conductivity, which strongly depends on soil water content, texture and structure (Vereecken et al., 2015). The hydraulic conductivity of a drying soil decreases by orders of magnitude, relative to a saturated soil, limiting the water movement from the bulk soil to the soil-root interface (Gardner, 1960). The second property that affects the flow rate limitation is the distribution of roots. The number of roots in each soil layer defines the length of the pathway (Gardner, 1964), with shorter pathways resulting in higher plant water availability. The third property defining the readily available water is the plant hydraulic conductance (Couvreur et al., 2014a). The maximal water flow rate that can be sent to the shoot to supply transpiration is limited by plant hydraulic conductance, which is mainly controlled by root radial conductivity (Couvreur et al., 2012; Hachez et al., 2012) and total root length (Alsina et al., 2011), though cavitation may limit the axial transfer of water under drought (Sperry et al., 2008).

While root growth affects plant water availability as mentioned above, soil water content can, in turn, affect root growth in many ways. A first feedback is the closure of stomata in conditions of low soil water availability, which limits photosynthesis and thus decreases the amount of carbon available to be invested in root biomass (Jackson et al., 2000). In tress, the higher root-to-shoot ratios and rooting depth, and the decrease of the biomass of fine roots and root length under water deficit it's well documented in field and laboratory experiments (Hartmann, 2011; Kozlowski and Pallardy, 2002; Mainiero and Kazda, 2006; Mokany et al., 2006; Poorter et al., 2012). Accordingly, the growth response is strongly influenced by the severity of the stress (Zang et al., 2014). Even a considerable amount of the available energy is invested to the growth of new roots, these young roots take up water more efficiently representing a suitable plant strategy under water deficit (Arend et al., 2011). However, other root traits, such as root density, specific root length and root area are only slightly affected (Eissenstat et al., 2000). Also, both high and low soil water contents limit root growth; the former through hypoxia and the latter through soil mechanical impedance (Bengough et al., 2006). Finally, soil water potential and soil temperature appear to be major factors influencing root growth (Teskey and Hinckley, 2006). Otherwise, at canopy level, many plant physiological processes may be related to the control of water status, and the shifting in isotope composition of plant compounds have been related as an interesting plant signaling of water stress, and described as a different approach for measurement of drought impact on the terrestrial ecosystems (i.e., oxygen, hydrogen and carbon isotope composition of plant tissues) (Burgess and Huang, 2014; De Jong Van Lier et al., 2006; Maxwell et al., 2014; Sun et al., 2011).

The understanding of processes affecting plant water availability has fundamental and applied implications. Recent studies have recognized the key role of roots in promoting acclimation to different types of stress; mainly through preferential growth and control of hydraulic properties that regulate transpiration (Alsina et al., 2011; Schoppach et al., 2014; Silva et al., 2015b). A better understanding of root response is, therefore, key for understanding water fluxes through the soil-plant-atmosphere continuum. Accordingly, here we examine the effect of root growth and plant hydraulic conductance on water availability for canopy transpiration of young walnut trees (*J. regia*) under different levels of water stress.

2. Materials and methods

2.1. Growth conditions and treatments in the greenhouse

The study was conducted from April 2015 to July 2015, using nine 8-month-old potted walnut trees (*J. regia*) cv. Chandler, grafted onto Paradox rootstock (*J. hindsii* x *J. regia*) in an experimental greenhouse at the University of California, Davis. Plants were grown in 0.02 m^3 pots filled with a 1:3 (v/v) mixture of a fine sand and organic compost. As the experiment was conducted over a short period and the plants were young, the size of the pots was considered suitable. Pots were kept covered with aluminum foil to avoid soil evaporation and their transparent walls were covered with plastic sheets that were black inside and white outside, to protect roots from light exposure. All plants received equal daily fertilizer application in water (6 ppm of NH_4^+, 96 ppm of NO_3^-, 26 ppm of P, 124 ppm of K, 90 ppm of Ca, 24 ppm of Mg, 16 ppm of S, 1.6 ppm of Fe; and < 1 ppm of Mn, B, Vu, Zn and Mo) (DANR/UC, 2014). Irrigation treatments were defined relative to each plant's "standard daily transpiration" observed under field capacity conditions one day before the experiment began (TD*) normalized to the control for that date and on subsequent dates (see below): (i) 100% of TD* (no water limitation) (T100); (ii) 75% of TD* (moderate water limitation) (T75) and (iii) 50% of TD* (strong water limitation) (T50) for a period of 10-days over three different time periods (April, April/ May, and July). All pots were maintained at field capacity for at least a week (any water excess was drained from the bottom of the pot) before the beginning of each 10-days period experiment. Replicates were monitored over time due to the careful tracking of soil-plant properties and limited availability of leaf psychrometers and high precision weighing scales for all individuals. Hence, the experiment was replicated using three different plants per treatment monitored over 10-days in three different time periods (April, April/May, and July), for a total of nine receiving one of the irrigation treatments and three control plants. While temporal replications integrate the effect of different insolation and temperature conditions in the greenhouse at each 10-day sampling event, we expect to observe consistent shifts between T100, T75, T50 throughout the experiment.

2.2. Soil-plant water status monitoring

Stem water potential (ψ_{stem}) was measured on expanded terminal leaflets located close to the trunk, every 15 min and averaged to hourly values, with a psychrometer/hygrometer (one per plant), model PSY-1 (ICT International Pty, Australia). The leaflet equipped with the psychrometer was fully covered with an insulation capsule limiting temperature fluctuations (see Fig. 1). As the monitored leaf did not transpire, the measurement was representative of stem rather than leaf water potential. An independent measurement of stem water potential was carried out weekly on fully expanded leaflets with a pressure chamber (PMS Instrument Company, Albany, OR). Prior to this destructive measurement, leaflets were enclosed in foil-laminate bags for at least 10 min (Fulton et al., 2001).

Plant transpiration rate (*TR*) was quantified by automatic weighing of pots on a high precision weighing scale (0.001 kg, Mettler Toledo PBA430) every ten minutes, averaged to hourly values. Draining water was collected daily in plastic reservoirs attached laterally to the bottom of the pots by flexible rubber tubing. Hence, the weight of leaching water did not affect the weighing scale reading until its collection. Both