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Transpiration of hazelnut
(*Corylus avellana* L.) orchards
in response to different climatic
conditions and implications for
water management





UNIVERSITÀ
DEGLI STUDI
DI PADOVA

Administrative unit: University of Padova

Department: Land, Environment, Agriculture and Forestry (LEAF)

PhD Program: Land, Environment, Resources and Health (LERH)

Batch: XXXII

THESIS TITLE

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Thesis financially supported by: Ferrero Trading Lux

PhD Program Coordinator: Prof. Davide Matteo Pettenella

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UNIVERSITÀ
DEGLI STUDI
DI PADOVA

Sede amministrativa: Università di Padova

Dipartimento: Territorio e sistemi agroforestali (TESAF)

Corso di Dottorato di Ricerca: Land, Environment, Resources and Health (LERH)

Ciclo: XXXII

TITOLO TESI

Risposte traspirative di corileti (*Corylus avellana* L.) in diversi contesti climatici e implicazioni per la gestione idrica

Tesi redatta con il contributo finanziario di: Ferrero Trading Lux

Coordinatore: Prof. Davide Matteo Pettenella

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SOMMARIO

Le piante sono indiscutibilmente alla base della vita umana poiché sono direttamente o indirettamente fonte alimentare, ma il settore agricolo pesa per più dell'80% sul consumo di acqua. La produzione agricola non può prescindere dall'apporto idrico per raggiungere le richieste del mercato, mentre deve fronteggiare una crescente scarsità di acqua, eventi siccitosi e ondate di calore. In questo senso, diviene fondamentale gestire le risorse idriche in modo sostenibile comprendendo le risposte idriche delle specie ai diversi climi.

Il nocciolo è un albero da frutto in grande espansione, che si è oggi affermato in tutti i continenti in risposta alla richiesta di nocciole da parte dell'industria alimentare. I noccioli debbono al contempo acclimatarsi alle nuove aree e rimanere produttivi. In questa situazione non c'è ancora una precisa conoscenza rispetto alla traspirazione media di noccioli irrigati e quindi alle loro necessità irrigue calate nel contesto climatico. I pochi studi sull'utilizzo dell'acqua nel nocciolo rimangono relativi alle dinamiche fogliari o sono difficili da trasferire agli agricoltori.

Lo scopo di questa ricerca verte su tre scopi principali nella prospettiva di essere un riferimento per i gestori dei corileti che operano in diversi contesti climatici: I) Identificare la risposta traspirativa di piante adulte in diversi climi, irrigazioni e cultivar. II) Stimare la traspirazione al livello di albero e, sulla base di ciò, proporre un approccio realistico per reintegrare le perdite traspirative volute ai corilicoltori. III) Esplorare le connessioni e le limitazioni alla traspirazione e ai processi di acquisizione del carbonio.

Si è installato un esperimento a lungo termine attraverso tre stagioni vegetative (2016-2019) in otto diversi corileti in Cile, Australia, Italia, Francia e Repubblica della Georgia, coinvolgendo due cultivar: Tonda Gentile delle Langhe and Tonda di Giffoni. Il flusso di linfa è stato monitorato con delle sonde a dissipazione termica di Granier (TDP). Sono stati misurati parametri meteorologici, contenuto idrico del suolo e parametri biometrici dei corileti. I TDP sono stati calibrati attraverso un misuratore di flusso e un approccio potometrico.

Le risposte traspirative si sono dimostrate linearmente correlate con il deficit di pressione di vapore atmosferico (VPD) fino a circa 20 hPa. L'intensità di radiazione si è anche rivelata importante nel diversificare le risposte tra i due emisferi, ma la cultivar e le diverse strategie irrigue hanno avuto un effetto irrilevante nel diversificare le risposte traspirative. La calibrazione dei TDP ha condotto alla correzione dell'equazione di Granier con parametri adatti al nocciolo. Questo, insieme alla stima dell'area fogliare ha permesso di individuare un intervallo di traspirazione per unità di area fogliare (1-2.5 l m⁻² giorno⁻¹). Tale risposta è alla base di una strategia di reintegro delle perdite traspirative che utilizza il VPD medio come principale predittore della traspirazione totale giornaliera. Il legame tra traspirazione e assimilazione potenziale di carbonio è stato considerato per far emergere l'intervallo di VPD in cui la conduttanza di chioma è massimizzata, e quindi, anche il potenziale di assimilazione.

Uno studio ancillare conclusivo mostra che per il riempimento delle nocchie la specie utilizza anche carboidrati precedentemente immagazzinati nel fusto oltre che a quelli forniti dalle foglie dell'anno.

SUMMARY

Plants are unquestionably at the basis of human life as they are directly or indirectly a source of food, but the agricultural sector accounts for more than 80% of water consumption. Agricultural production cannot ignore water supply to meet market demands, while it faces growing water shortages, droughts and heat waves. In this sense, it is paramount to manage water resources in a sustainable way by understanding the water responses of species to different climates.

The European hazelnut is an expanding orchard tree, which has now established in all continents to answer the increasing demand for nuts from the food industry. Trees face the challenge of acclimating to new areas, while remaining productive. In this situation, still no precise knowledge exists about the average transpiration of hazelnut orchard under standard irrigation and thus about the real irrigation needs respect to its environment. The few studies on hazelnut water use are mainly leaf-based or hardly transferred to growers.

This research focuses on three main topics in the perspective of providing useful reference for orchard managers set in different climatic contexts: I) to identify the pattern of transpiration response to different climates, irrigation and cultivars. II) To estimate the transpiration at the tree level and propose a realistic tool for growers to reintegrate water loss. III) Explore the links and limitations of transpiration activity and carbon gain processes.

We set a long-term experiment across three growing seasons (from 2016 to 2019) on eight orchards in Chile, Australia, Italy, France and Republic of Georgia, involving two cultivars: Tonda Gentile delle Langhe and Tonda di Giffoni. We monitored in continuous the sap flow with Granier's thermal dissipation probes (TDPs), the meteorological parameters and the soil water content. Tree biometrics and orchard features were recorded as well. TDPs were calibrated with a liquid flow meter applied to a potometric system.

The transpiration responses showed to be linearly correlated to the vapor pressure deficit of the atmosphere (VPD) until 20hPa. The radiation intensity produced different responses in the two hemisphere, but cultivar and doubled irrigation had little impact. The calibration of

TDPs lead to the correction of the Granier's equation with parameters suitable for hazelnut. This, together with the estimation of the tree leaf area, allowed to derive a range of transpiration per unit of leaf ($1-2.55 \text{ l m}^{-2}\text{day}^{-1}$). This is the basis for a water loss reintegration strategy by knowing the mean daily VPD. The link between transpiration and carbon assimilation was considered to highlight the interval of VPD at which the canopy conductance, and thus the potential carbon assimilation, is maximized. A conclusive ancillary study shows through the manipulation of C sources and sinks that the nut production depends on C previously stored in the stem showing that branches are not fully carbon autonomous.

INTRODUCTION

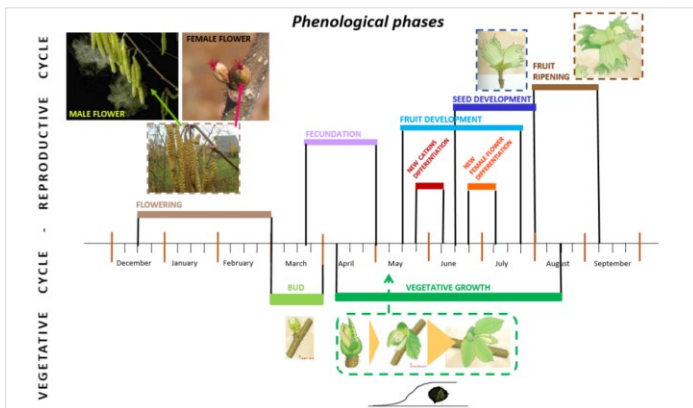
Water use and availability in orchards remains a pivotal issue being the most important limiting factor for agricultural production and food security (de Fraiture and Wichelns, 2010). In particular, many new target areas for orchard plantation are located in correspondence with areas prone to severe water scarcity (OECD - Organisation for Economic Cooperation and Development, 2017). The agricultural sector has the responsibility to find concrete solutions to limit fresh water waste, since it accounts for the largest share for water consumption in all world countries (Hoekstra and Mekonnen, 2012; Tilman et al., 2001). In this context, the long term planning of irrigation strategies at global and local scale will become challenging but paramount at the same time. Moreover, in countries as Australia, irrigation water must be quantified one or two years in advance to by water use rights to the State. In this context, the awareness of irrigation demand becomes even more necessary. In this perspective, a deeper understanding of water use of expanding crops in diverse climatic conditions can help in refining its management according to its acclimation capacity.

European hazelnut is becoming an increasingly appreciated species for its nut production. At present, it is one of the most important nut crops in the world: FAO estimated that hazel planted crops had a 13.9% global increase in the last decade, for an amount of around 800.000 Mg ha⁻¹ at the global scale (FAOstat, 2014). In Italy a national plan was launched in 2018 to plant 20.000 new hectares of hazelnut orchards by 2025, i.e. +30% of the actual surface (Tosi, 2018).

The orchard cultivation of this forest species is traditionally set in the Mediterranean area, especially Turkey, Italy and Spain. Orchards on the Black Sea region are on steep hill slopes, with low fertility, shrub habit of the trees, moderately dense (600/700 trees ha⁻¹) and not irrigated. There, the climatic conditions have been ideal up to now: temperate climate with frequent precipitation events and moderate summer temperatures (Me and Valentini, 2006). In other areas of Turkey, Spain or Italy water provision is often a challenge because total precipitation is below hazelnut requirements (800 mm year⁻¹). In order to enlarge the production and make it less dependent from one single country (Turkey produces between 70 and 80% of the world nut yield), investors are experimenting hazelnut cultivation outside native areas.

Introduction

One of the most effective attempts occurred in Chile, where since 25 years the European hazelnut has been introduced reaching up to 4.100 ha of orchard in Fruticola Agrichile, and 20.000 ha more from the contribution of out growers. Other examples of extensive orchards are in Australia, South Africa, Butahan, Serbia, that are new targets where hazelnut is spreading for commercial purposes (Corte et al., 2009; Solar and Stampar, 2011). However new sites often have soil quality limited by low nutrient availability, intense light radiation and severe climatic conditions (i.e. high atmospheric evaporative demand, insufficient precipitation, thermic excursions, lack of chilling hours). In these contexts, the economic profitability of cultivating this species under diverse climatic conditions depends on the capacity to mitigate non-desirable climatic effects, and especially to manage water sustainably while ensuring profitable yield, which is around 3-5 Kg tree⁻¹ for the most appreciated cultivars Tonda di Giffoni and Tonda delle Langhe (Cristofori et al., 2014; Solar and Stampar, 2011). The most sensitive stages of the hazelnut phenology (Figure 1) to climatic condition are the flowering and pollination stage (Ascari et al., 2018) and the kernel filling period (seed development-fruit ripening). While the lack of proper chill hours or frost excess are a triggering factor for the pollination process (Mehlenbacher, 1991), water availability affects the final yield (Marsal et al., 1997).



Phenological phases of hazelnut. Picture adapted from Bignami et al. (1999) and Ferrero Hazelnut Company archive material. Information are for the species in general. More detailed cultivar features can be found in Solar and Stampar, 2011.

The European hazelnut (*Corylus avellana* L.) is a deciduous broadleaf species growing mainly with a multi-stem habit. Its native ecosystem is the understory of temperate forests of the northern hemisphere. Thus, a temperate climate is ideal for its growth (Tombesi, 1991), with average temperatures between 12°C and 16°C and maximum temperature not exceeding 36°C. It can survive without damage down to -15°C, while chill hours are a paramount condition for gametic reproduction. Chill hours range between 100 and 1200 h, depending on the type of flower (male or female) or vegetative bud, as well as from the cultivar. Ideal precipitation amount is between 800 and 1000 mm year⁻¹, well distributed during the summer period (Fideghelli and De Salvador, 2009). It prefers neutral or slightly acidic soils (pH = 5.5-5.6). Well-drained and fertile soils are ideal, but it can also succeed on a wide variety of different substrata (Gispert et al., 2005). The root system is shallow and constituted by fine roots that remain within the first 50-60 cm of the soil (Roversi, 2001). However, recent observations with field rhizotron in Chilean orchards revealed root depth on adult trees down to 80-100 cm depth (data not published). The reproduction occurs either in agamic way through sprouts from the stump or in a gametic way via wind transported pollen between December and February according to the cultivar. In orchards, rooted suckers are the most used propagation technique (Fideghelli and De Salvador, 2009), while tree management is mainly based on sprouts selection from the stump, so that the shrub habit remains the predominant training in productive areas. A peculiar trait of hazelnut is the synchronous alternate bearing in the nut production (1-2 years) typical of many nut and fruit trees (e.g. olive - Bustan et al., 2011). Many plants oscillate between years with abundant seed production and other very scarce with few years characterized by average seed amounts. The genetic selection has not been so strong yet to mitigate this trait.

In general hazelnut is considered a water saver pioneer species because of the strong reduction of stomatal conductance and assimilation in conditions of moderate soil water deficit and low atmospheric humidity (Baldwin et al., 2003; Trotter, 1951). Schulze & Kueppers (1979) demonstrated that high vapor pressure deficit (VPD) has a detrimental effect in reducing the transpiration and thus carbon uptake, especially when coupled with dry winds. Marsal et al. (1997) confirmed the negative effect on transpiration of high VPD combined with scarce soil water availability. Stomata close since early hours of the day (e.g. 9-10

AM), while sap flow flattens already at 5-6 hPa in rain-fed orchards in the North-West of Italy (Pisetta 2012), as often observed in water saver species. Also, in wild individuals, Hogg et al. (2000) reported severe inhibition of stomata conductance by vapor pressure deficit greater than 5 hPa in forest understory.

The above-mentioned studies depict hazelnut as a very sensitive species for what concerns the evaporative demand of the atmosphere, while a pioneer species for what concerns its capacity to establish just after a disturbance.

High temperatures ($>30^{\circ}\text{C}$), high light intensity ($>1000 \mu\text{mol m}^{-2} \text{s}^{-1}$) and high vapor pressure deficit ($>20 \text{ hPa}$) affect stomatal conductance (g_s) relevantly (Tombesi, 1994; Awada et al., 2003; Girona et al. 1994; Hogg et al. 2000). Awada and Josiah (2007), showed similar results on a field study where stomatal conductance and photosynthesis occurred independently from irrigation treatment, suggesting that soil water status is not sufficient for explaining stomatal response to drought. At the same time, strategies of deficit irrigation management for hazelnut are still under discussion because of the controversial effects on nut yield (Marsal et al., 1997).

Relatively to light demand, hazelnut seems to tolerate low light regimes (less than 10% of full sunlight), but can grow under full sunlight. Hazelnut is claimed to maximize leaf gas exchange (both carbon gain and water loss) at low light ($300 \mu\text{mol m}^{-2} \text{s}^{-1}$), according to von Stamm (1994) and Hogg et al. (2000). However, more recent results (Tombesi et al., 2015) show light saturation at about $650\text{-}700 \mu\text{mol m}^{-2} \text{s}^{-1}$ for young seedlings. In coping with greater light intensities, hazelnut appears able to modify leaf structure, chemistry and, to some extent, photosynthetic physiology (Catonì et al., 2014). In this sense, hazelnut might be considered to have a certain degree of plasticity in response to light variation. A recent work on seedling from different hazelnut cultivars (Cincera et al., 2018) claimed that the difference in genotypes may play a significant role in the acclimation to new environments, because they show different stomatal sensitivity to VPD in a controlled environment.

To individuate the limiting factors for the species, allow to focus on the best conditions leading to optimize gas exchanges. Carbon assimilation is indeed particularly important in a productive context, because, on the long term, it constitutes the pool for building the reproductive biomass, i.e. nuts.

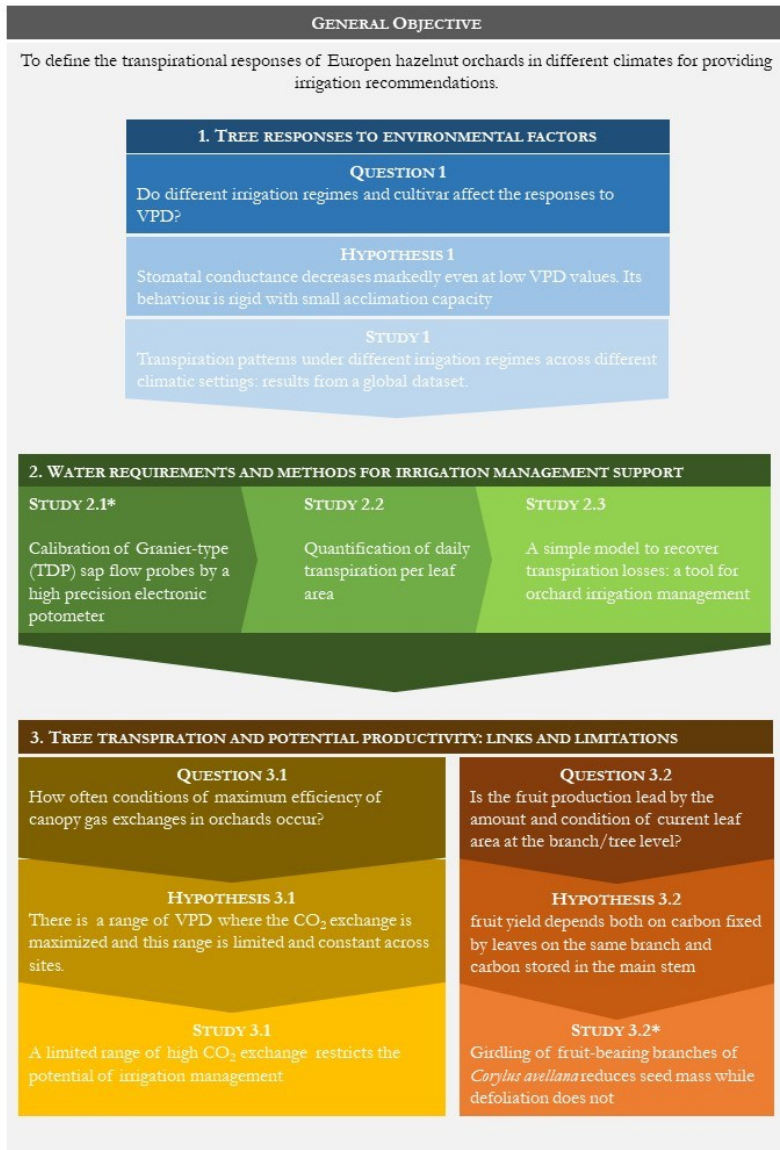
From the literature on nut-producing trees, emerges that carbon reserves devolved to fruit production would be exclusively supplied from current photoassimilates. (Hoch et al., 2013). Therefore, current water uptake might be a proxy for nut productivity, because the water vapor conductance is linked to the carbon uptake. The intrinsic Water Use Efficiency – carbon gain per unit water loss (iWUE) was estimated to range between 30 and 50 $\mu\text{mol mol}^{-1}$ in hazelnut (von Stamm, 1994; Awada and Josiah, 2007), that is considered to be relatively low in comparison to other cultivated woody species across the globe (Adams et al., 2016).

Despite all these findings, the information gathered on the behavior of mature trees in the field remains little and scarcely applicable by growers, who need to shed light on the costs-benefits of their orchards in terms of water consumption.

Therefore, the aim of this work is to set the basis to provide physiologically based information for a large audience of growers who act in very different climatic settings.

Here I will try to address a set of interlinked issues: i) How to identify a specific pattern of transpiration response for the species. ii) What is the impact of different irrigation strategies and cultivar on the transpiration activity. iii) How to quantify the transpiration at the whole tree level and provide an easy tool for growers to reintegrate the water lost by transpiration. iv) How to use the stomatal behavior for predicting the potential limitations to productivity in different environmental conditions.

AIMS & HYPOTHESES



STRUCTURE OF THE THESIS

The main aim of this study was to characterize the transpiration responses of *Corylus avellana* (L.) in productive contexts located in different climatic areas and to highlight the implications for orchard irrigation management.

A general introduction with an overview of the study areas opens the manuscript. Because the project is focused on a single species on several study areas, it is presented in a mixed form that consists of chapters on specific topics. Prodrormal or complementary subsections are inserted in the form of published papers. Each chapter follows the form of a paper with a short introduction and conclusions.

Chapter 1. Tree responses to environmental factors describes the pattern of variation of sap flow to VPD. The effects of irrigation treatments and cultivar on these patterns across 8 world-wide distributed sites are described.

Chapter 2. Water requirements and methods for irrigation management support:

Ch. 2.1 Calibration of Granier-type (TDP) sap flow probes presents the results of “Calibration of Granier-type (TDP) sap flow probes by a high precision electronic potometer” published in *Sensors* as a method paper. It presents an innovative technique of calibration that combines a high precision potometer and a liquid flow meter. A corrected equation for TDP specific for European hazelnut orchards is proposed here and is prodrormal to quantify the transpiration.

Ch. 2.2 Quantification of daily transpiration at the level of leaf area unit in European hazelnut plantations provides a quantitative estimation of transpiration losses based on biometric data of world-wide distributed productive orchards. An accessible model to calibrate the irrigation needs based on basic meteorological parameters is proposed

Chapter 3. Tree transpiration and potential productivity: links and limitations aims at connecting diurnal transpiration to the potential CO₂ exchange:

Ch. 3.1 A limited window of high canopy conductance efficiency analyzes the canopy conductance as a proxy for CO₂ exchange and the conditions in which it is maximized.

Ch. 3.2 Manipulating C sources and sinks and effects on nut production presents the results of the paper “Girdling of fruit-bearing branches

of *Corylus avellana* reduces seed mass while defoliation does not” published in *Scientia Horticulturae*. It was designed, to explore the correlation and role of the branch and tree leaf area as well as stem reserves to the fruit production in hazelnut, an alternate bearing species.

A final chapter with overall conclusions summarizes the work findings.

STUDY AREAS

A long-term field study was conducted in eight worldwide-distributed orchards (Figure I; detailed description in Table I) of European hazelnut (*Corylus avellana* L.) in two main commercial cultivars: Tonda Gentile delle Langhe (TGL) and Tonda di Giffoni (TG). All orchards are subject to standard agronomic practices to lead trees to the nut production. The standard fertilization plan for production consists in 80-100 units of N, 25-40 units of P and 60-80 units of K, distributed 1/3 in autumn and 2/3 in spring time, at the bud break stage. Pruning is both manual and mechanical, while suckers control occurs twice a year by chemical applications. The irrigation is organized as raised driplines (0.5-1 m from the ground) to allow for an easier mechanization at the harvesting stage. Weed control occurs by chemicals *intra-rows* and mechanically in *inter-rows*. Pest management is necessary in most of the areas to control a variety of insects that affect severely the nut production reaching up to 100% yield loss in some cases. The most important are the stink bugs, Pentatomidae Family, (*Gonocerus acuteangulatus* Goeze, *Palomena prasina* L., *Nezara viridula* L. and *Halyomorpha halys* Stål) and the weevil (*Curculio nucum* L.), that cause deterioration and bitterness of fruits as well as traumatic abortion. Pest and disease treatments are organized as 1 treatment per year against eriophyids; 2 treatment per year with fungicide/bactericide and up to 5 treatment per year against *Halyomorpha halys*, which expanded massively in the last years, mainly in European-Caucasian areas (Maistrello et al., 2018; Musolin et al., 2018). The harvesting is performed mechanically in all monitored areas.

Orchards belong either to large farms Fruticola Agrichile, Agri Australis and Agrigeorgia, with a cumulative orchard extension up to 4.100ha in Chile, as well as to medium-small private owners in Italy and France (2 to 5 ha).

Study areas

Table I. Summary of the Geographic localization of monitoring sites and other features. Treatment codes *std*, *std*2*, *std*0.5* refer to the variation respect to the standard irrigation (e.g. *std*2* = 200% *std* irrigation), *sp*=*sprinklers*.

Site	Country	Site Name	Coordinates		Cultivar	Treatment	Monitoring period
			N	E			
NAR	Australia (NSW)	Narrandera	-34.81596	146.6792	TG	<i>std</i>	2017-2019
ORG		Orange	-33.32562	149.08358	TG	<i>std/std+sp</i>	2017-2019
CAM	Chile (Maule)	Camarico	-35.30578	-71.35931	TG	<i>std/std*0.5</i>	2016-2019
SSEB		S.Sebastian	- 35.29978	-71.5468	TGL	<i>std/std*2</i>	2016-2019
GEJ	Republic of Georgia	Gejeti	42.319013	42.209547	TG	<i>std/std*0.5</i>	2016-2019
CHI		Chitaskari	42.461852	41.853365	TG	<i>std</i>	2016-2019
FRA	France (Bordeaux)	Cancon	44.302950	0.571625	TG	<i>std/std*0.5</i>	2016-2019
ITA	Italia (Piemonte)	Baldissero d'Alba	44.755235	7.917502	TGL	no irr	2017-2018

Sample plots included 15 trees (Figure II), six of which were equipped with a set of self-made (TESAF – UniPD) thermal dissipation probes (TDPs) for sap flow record.

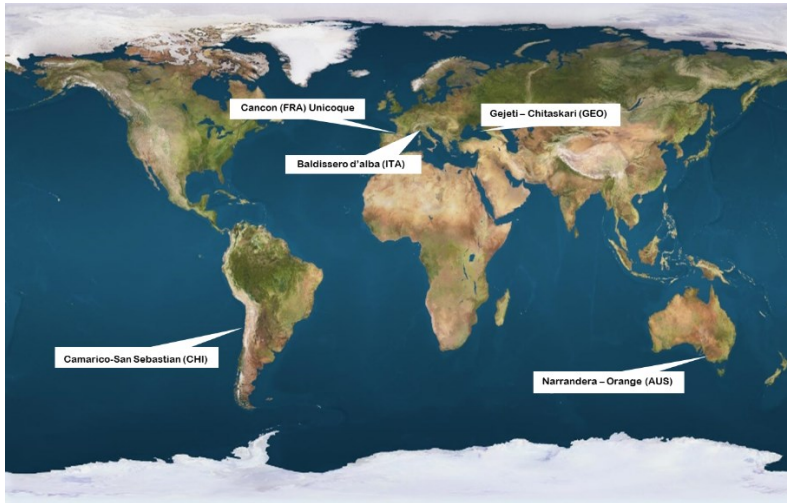


Figure I. Map of the geographic location of the monitoring site.

Probes are 20mm long according to the original Granier's design. TDPs were set North-East facing and thermally insulated with aluminum heat insulation foil and styrofoam. A set of two dendrometers and soil water content probes (TDR) completed the site equipment. Diameter, height and crown radius were recorded for all main sprouts belonging to monitored trees (Table II). A data logger (CR1000, Campbell Scientific, Inc., USA) recorded all data every 15 minutes along the growing season. An example of sample plot is reported in Figure II. A solar panel and a battery provided constant power supply to the station. Air temperature (°C) and relative humidity (RH%) have been recorded right on top of tree crowns in the orchard. A double check on meteorological parameters was performed comparing T°C and RH with farm or regional weather stations positioned few km far from the orchards at 10m height.

Table II. Biometric data at each site. Values are reported as means of trees included in the plots. S.d. in brackets.

Site	Orch. density (n. ha ⁻¹)	Tree height (m)	Crown radius (m)	Sprout n. (n.)	Sprout diameter (cm)	Basal area (dm ²)	Tree leaf area (m ²)
CAM	667	2.8 (0.27)	1.81 (0.21)	7.0 (1.10)	5.70 (1.13)	1.87 (0.16)	54 (4.77)
CHIT	667	5.1 (0.15)	1.78 (0.15)	10.20 (1.92)	4.97 (0.96)	2.33 (0.54)	99 (24.58)
FRA	667	5.2 (0.27)	1.5 (0.15)	3.11 (0.88)	10.23 (2.12)	2.65 (0.37)	91 (13.51)
GEJ	667	3.5 (0.8)	1.45 (0.23)	11 (3.35)	3.10 (0.65)	0.87 (0.27)	23 (7.17)
ITA	400	3.8 (0.12)	2.18 (0.17)	6.5 (1.64)	6.33 (1.62)	2.44 (0.54)	77 (18.46)
NAR	500	2.1 (0.15)	1.26 (0.22)	7.5 (2.07)	2.29 (0.57)	0.53 (0.10)	14 (2.39)
ORG	500	3.2 (0.03)	1.31 (0.14)	4.3 (0.51)	5.20 (0.68)	0.94 (0.09)	27 (2.72)
SEB	667	4.5 (0.12)	2.40 (0.18)	5.8 (1.72)	6.27 (3.12)	2.23 (0.24)	73 (9.13)

Study areas

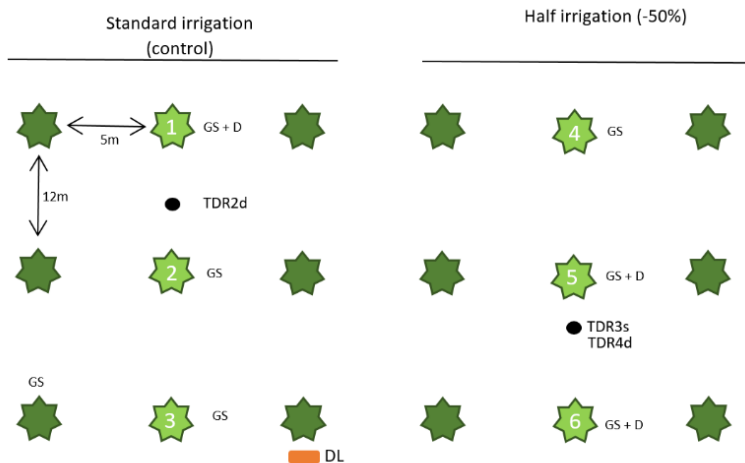


Figure II. Example of a sample plot in Camarico-Chile. Numbered trees are monitored subjects for the sap flow with Granier sensors (GS) and Dendrometers (D). Soil water content probe (TDR) is shown in correspondence with black dots. At the datalogger (DL) all sensors converge. $T^{\circ}C$ and RH%, are measured in correspondence with DL, right above tree crowns .

Plots were installed in commercial orchards with various tree spacing: 5x4, 5x3 and 5x6. Trees grew as either free vase or single stem tree in two different commercial varieties : Tonda Gentile delle Langhe –TGL and Tonda di Giffoni-TG. All sites were provided with drip irrigation. The irrigation treatments are different across sites as shown in Table I, and include full standard irrigation on single drip line (std) (ET_c calculated by local agronomists), double irrigation realized through a double drip line (std*2), half irrigation with narrower nozzles (std*0.5), no irrigation (no irr). In Australia-Narrandera site, the standard irrigation was further supported with ground-based sprinklers (std + sp). The experimentation started in 2015 in Australia and continued in all sites for three years. Every treatment included three replicates. Sap flow probes were replaced every year to prevent the deterioration of the signal (Moore et al., 2010).

CHAPTER 1
TREE RESPONSES TO ENVIRONMENTAL
FACTORS

Introduction

Plants face a daily trade-off between access to carbon dioxide and dehydration risk. Indeed, the atmosphere is the source of carbon, the basis of photosynthesis, but also it is relatively dry respect to the mesophyll. There are two main levels at which plants regulate carbon assimilation and water loss: at whole-tree level or at the single-leaf level (Schulze et al., 1987). At the whole-tree, the transpiration response to the atmospheric environment expresses the capacity of a species to regulate water loss under specific atmospheric condition. However, it is still not clear if the transpiration response is a rigid trait in a species and on which extent it can be modified by genetic selection or management (e.g. soil water regulation).

In trees grown for productive purposes a strategy that favors carbon gain or water preservation is decisive to define new suitable sites to grow a species or to favor specific cultivars. Indeed, it emerged that the responses of different vine (Rogiers et al., 2009) or poplar genotypes (Attia et al., 2015) have different response capacity according to a more isohydric or anisohydric behavior. The isohydric behavior would lead to increased water use efficiency: the CO₂ assimilation for biomass production would be sacrificed in favor of preserving the water transport system (cavitation avoided). On the contrary, a more anisohydric behavior would favor CO₂ assimilation to the detriment of high transpiration and cavitation risks.

European hazelnut has been observed to be quite sensitive to high VPD (Cristofori et al., 2014; Girona et al., 1994; Schulze and Kueppers, 1979) showing a conservative behavior respect to water condition. Compared to other nut trees such as almond it presents a more isohydric behavior. Indeed, Marsal et al. (1997) showed that hazelnut tends to maintain a nearly constant Ψ_{leaf} despite soil dehydration and, thus, a higher relative water content by reducing the stomatal conductance. The isohydric behavior would lead to increased water use

efficiency: the CO₂ assimilation for biomass production would be sacrificed in favor of preserving the water transport system (cavitation avoided) (Attia et al., 2015). These responses allow to classify the hazelnut as a water saver species. The sensitivity to VPD in wild types of hazelnut is remarkable also when soil water is not limiting. Hogg et al. (2000) showed daytime stomatal conductance to be inversely correlated to VPD for values higher than 5 hPa. Tonda Gentile delle Langhe (TGL) in particular, is believed to be the most sensitive among hazelnut cultivars. Studies at the leaf level in nursery trees, reported TGL to suffer from large stomatal limitation at high VPD compared to others commercial cultivars such as Tonda Romana or Tonda Giffoni (Cincera et al., 2018). This might be one of the main causes to explain why TGL hardly acclimate to environments different from its native area (North Western Italy) (Cristofori et al., 2014; Solar and Stampar, 2011).

Often, the responses to environmental conditions can be investigated with high precision at the leaf level through leaf gas exchange measurements. However, on mature trees, canopies include a variety of leaf conditions (sun leaves-shade leaves, deep or superficial position in the crown) and many of these change in continuous during the day. Such a complexity is difficult to model in large trees, if reference observations are based on few leaves of small individuals where the exposure to radiation and VPD condition are even in the crown (e.g. small-young seedlings). Plant-based methods instead, have a great potential for irrigation control (Scholasch, 2018) because observations are more easily up-scaled. A tree based monitoring is perhaps more appropriate to analyze the responses of transpiration to environmental factors in orchards. Sap flow sensors, which are widely used in tree species, can get the average condition of the tree crown.

In this study, we analyzed the responses of the sap flow to VPD in eight orchards of European hazelnut. We hypothesized that the relative transpiration response to VPD has a similar pattern across sites under standard irrigation treatment. We expect that under the same irrigation

treatment the variation of the transpiration with VPD is relatively conservative across sites. If the species tends to preserve the leaf hydration, we also expect that, as soil water availability decreases, the response curve of sap flow to VPD shifts towards lower values (i.e. higher sensitivity). Thus, we tested the impact of the seasonal variation between the dry and wet months in each site. The effect of two cultivars and five irrigation treatments on the transpiration pattern was also analyzed.

Materials and Methods

Data preparation

The download of sap flow data from local data loggers was performed by a local farm responsible. Data were sent to our lab and went through a verification and normalization routine. The first step consisted in checking the data set, i.e. detecting errors or missing values. Anomalies in the records such as sudden drops of sap flow values, have been visually detected and removed when a cross check with meteorological data revealed the inconsistency in the sensor behavior (e.g. heavy rainfall). Small gaps in the dataset (few cells) have been reconstructed through correlations with other probes or meteorological data, while gaps of several days remained empty. Once the normalization was completed, the first elaboration step consisted in calculating the k value of the Granier's equation (Granier, 1985) for the sap flow density: $Fd = a \cdot k^b$. $K = (\Delta T_0 - \Delta T) / \Delta T$, where ΔT is the temperature difference between the two probes (the heated *vs.* the reference one), ΔT_0 is the maximum temperature difference (i.e., the condition corresponding to zero flow). In the original Granier's equation $b = 1.231$ and the constant $a = 4.284$ when Fd is expressed as $\text{dm}^3 \cdot \text{dm}^{-2} \cdot \text{h}^{-1}$. In order to compare trees and sites to search for differences in the responses we calculated the relative sap flow density. $F_{rel} = Fd / Fd_{90^\circ}$, where Fd_{90° is the value at which Fd reaches the 90th

quantile in the data series. Because the sampling frequency was high (every 15 minutes) and the data series long (3 growing seasons), we chose to select as maximum the 90th quantile instead of the absolute maximum in order to decrease the disturbance of single out layer observations.

Data sets from all sites and years were ordered according to a fixed scheme, which allows to aggregate, read and elaborate the variables in the software R. All data processing of the full dataset have been performed in the R version 3.5.3. Data were merged and prepared for the analysis stage.

Data analysis

Because we observed *Fdrel vs.* VPD class to remain linear until 20 hPa, we analyzed the differences between sites considering the early response for values <20hPa. *Fdrel* and VPD were normalized as logarithms to run a linear model and compare slopes and intercepts. The steeper the slope the most is the tree coupled to the atmosphere, the flatter the slope, the highest is the decoupling from the atmosphere, i.e. stomata tend to close.

We evaluated the influence of the site on the *Fdrel vs.* VPD relation. The variable site influenced significantly the pattern differences. Thus, a linear model is used to look at the pattern of variation between *Fdrel vs.* VPD within sites and between months under standard treatment. The same analysis was run to compare different irrigation treatments and cultivars.

Results and Discussion

Climatic conditions

A summary of the main meteorological parameters (Figures 1.1, 1.2; Table 1.1) and the soil water content variation (Figure 1.3) during the growing seasons is reported. The absolute maximum temperature reached 46.6°C in Narrandera, Australia (NAR) during January 2019, while the absolute minimum was still registered in Australia, but in the Orange site (ORG) with -0.5°C in November 2018

Table 1.1 Temperatures as daily means, daily maximum and absolute maximum; mean values (s.d.) of vapor pressure deficit VPD and soil water content VWC at each site during the growing season. The growing season here is considered as the most important month for the fruit development: May to August for Northern Hemisphere and November to February for the Southern Hemisphere. Global radiation data from NASA Prediction Of Worldwide Energy Resources (<https://power.larc.nasa.gov/>)

Site	T (°C)			VPD (hPa)	VWC (m ³ m ⁻³)	Global radiation (MJ m ⁻² day ⁻¹)
	Daily mean	Daily max	Abs max			
CAM	19.38	24.53	37.6	10.49 (2.54)	0.33 (0.05)	29.512
CHIT	22.07	27.12	40	4.57 (1.86)	0.39 (0.1)	19.432
FRA	20.63	26.71	37.9	8.25 (2.73)	0.4 (0.06)	19.562
GEJ	23.18	28.99	40.2	7.14 (2.91)	0.45 (0.04)	18.591
ITA	21.86	28.6	38.9	9.64 (3.66)	0.2 (0.07)	19.672
NAR	25.06	33.68	46.6	20.13 (7.12)	0.09 (0.03)	26.770
ORG	20.22	27.41	39.46	11.91 (4.88)	0.41 (0.02)	25.042
SEB	20.39	24.13	38.59	12.38 (2.76)	0.34 (0.06)	29.512

The correlation matrix with meteorological parameters revealed VPD as the most correlated variable with *Fd*. The soil water content was maintained close to the field capacity in the standard treatment. In the Italian site (ITA) with no irrigation, the soil water content refers only the precipitation. Because rainfall remained abundant during the Month of June, the very dry period at this site started from July onwards.

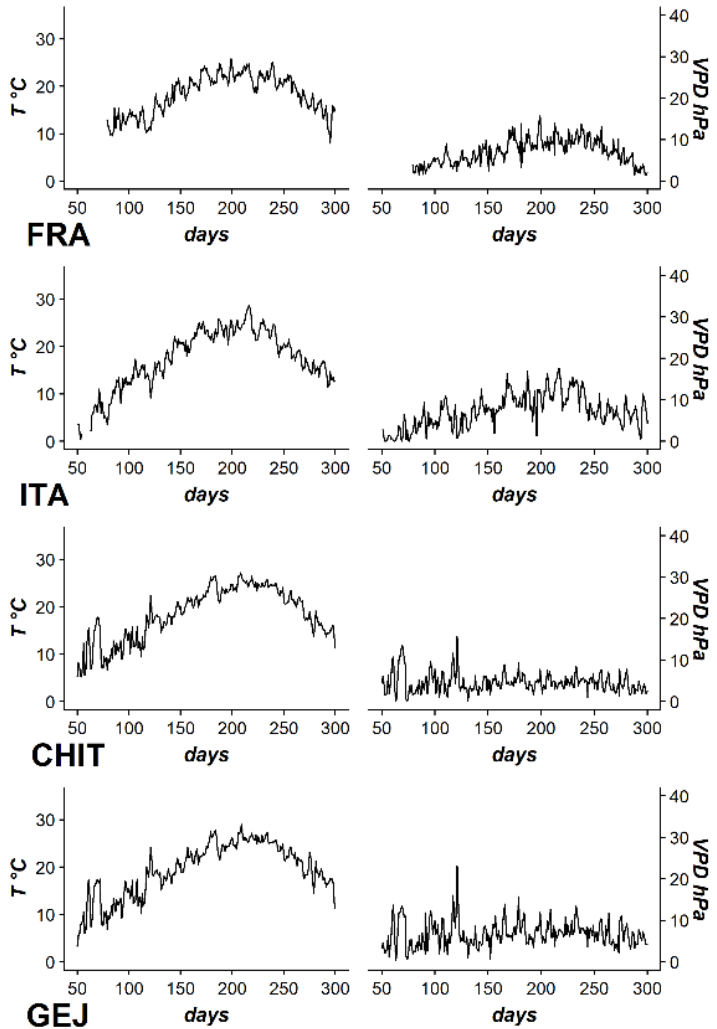


Figure 1.1 Climatic variables in the Northern Hemisphere. Daily means of 3 years data collection. Temperature in $^{\circ}\text{C}$ and vapor pressure deficit of the atmosphere (VPD) in hPa are reported as daily means. Days refer to the days of the year for boreal sites, while for the Southern Hemisphere they have been converted to Boreal days of the year to allow for a comparison across site

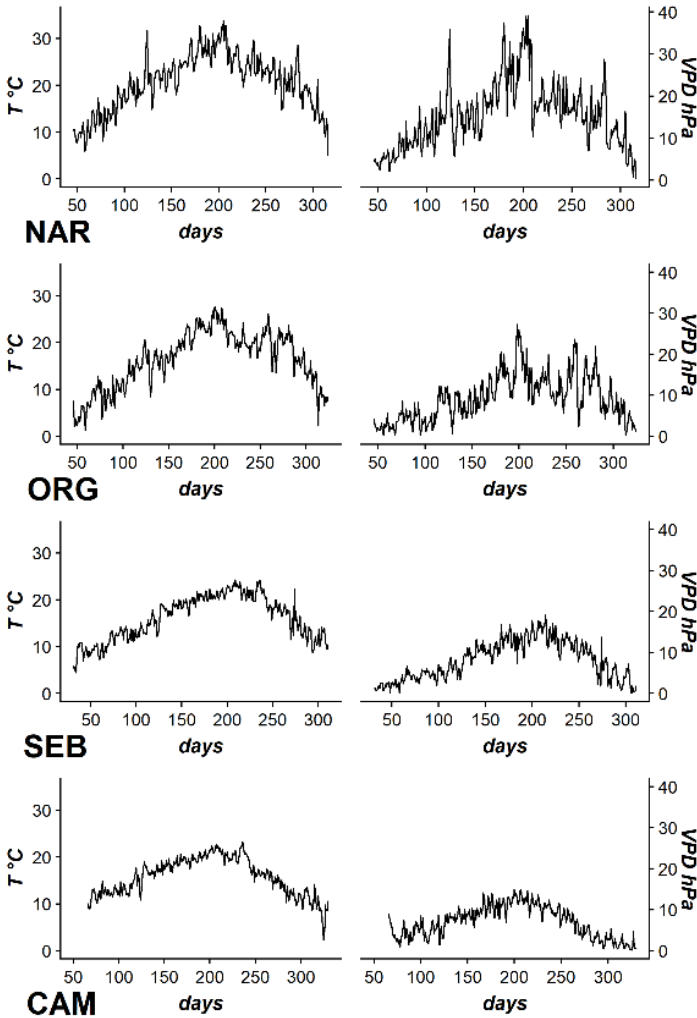


Figure 1.2 Climatic variables in the Southern Hemisphere. Daily means of 3 years data collection. Temperature in °C and vapor pressure deficit of the atmosphere (VPD) in hPa are reported as daily means. Days refer to the days of the year for boreal sites, while for the Southern Hemisphere they have been converted to Boreal days of the year to allow for a comparison across sites.

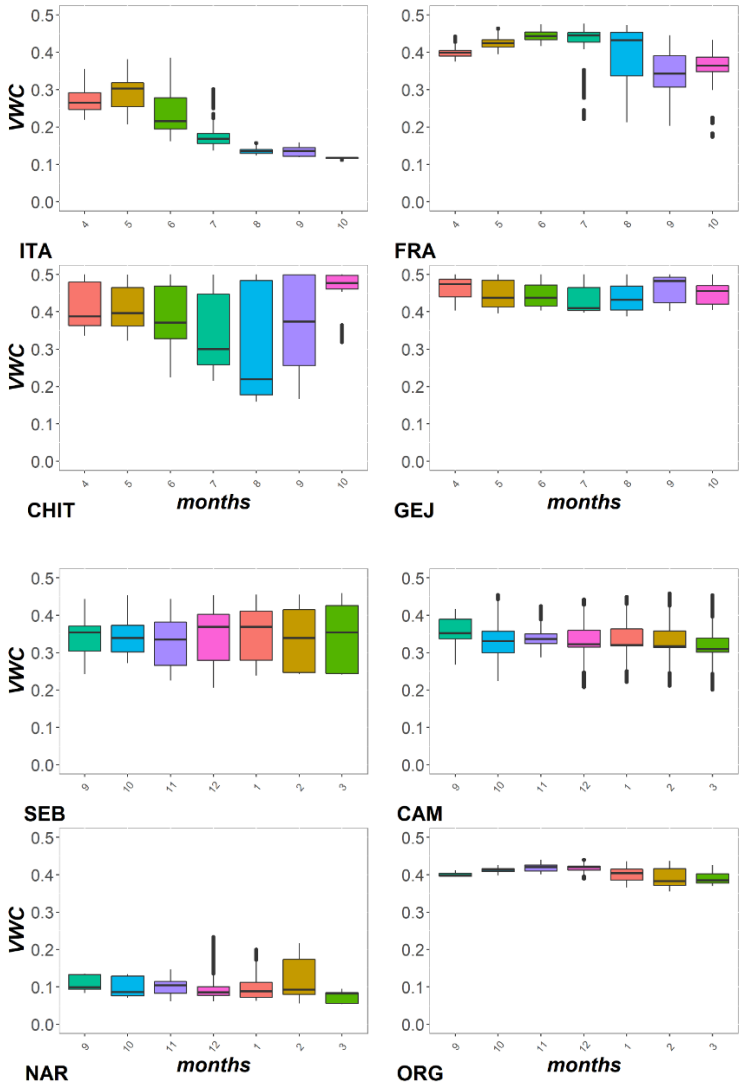


Figure 1.3 Volume of water content ($m^3 m^{-3}$) measured with the TDR at 35cm depth in standard irrigation treatments in each site and no irrigation in site ITA. Months are expressed as progressive numbers from January (1) to December (12) according to the growing season at each hemisphere. Northern sites in the first two rows, Southern sites in the 3rd and 4th rows. Site ID is reported in capital letters in the bottom left of each chart.

Site related differences

To explore site related differences we extracted only trees under standard irrigation. Fd_{rel} increases linearly with VPD until a bending point, and in general, it is similar for the most of the orchards in the study areas (Figure 1.4). The curve bends around 20hPa, after which it tends to flatten, i.e. the sap flow does not increase as quickly as before per unit of VPD. At the whole tree level the bending point occurs at lower VPD respect to what observed by Cincera et al. (2018) on leaves. Indeed, they reported the bending point at about 40 hPa for Tonda di Giffoni and Tonda Gentile delle Langhe.

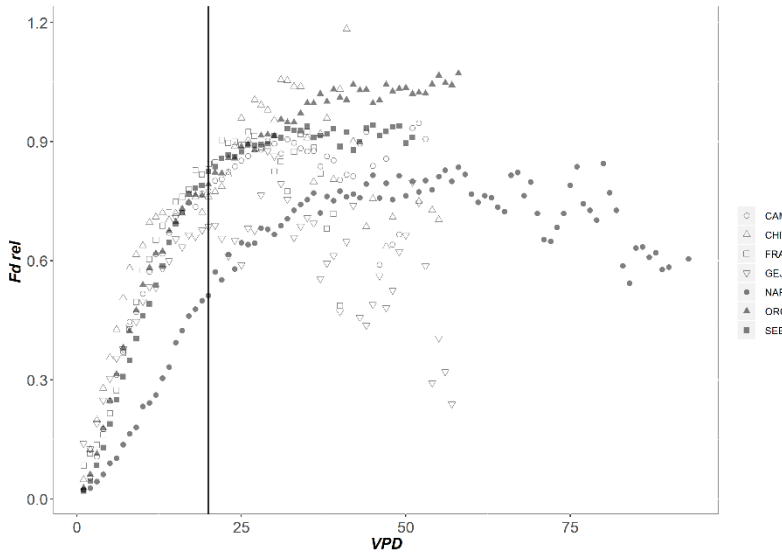


Figure 1.4 Relative sap flow Fd_{rel} vs. VPD classes in the 8 sites under standard irrigation. Each point is the average per VPD class.

From the analysis of the linear part of the curve on standard irrigated trees (Table 1.2) emerged that the intercept of the Australian site NAR is statistically different from all other sites. However, the slopes

emerged to be clustered in two groups: Georgian (GEJ and CHIT) sites and France (FRA) showed a significant difference in slope respect to the rest of the sites in the Southern hemisphere, included NAR. This leads to two observations: I) sites in the Southern hemisphere share a similar response Fd to VPD. This might be related to the higher global radiation in the Southern hemisphere during the growing season (on average $+8.64 \text{ MJ m}^{-2} \text{ day}^{-1}$), which increases the leaf temperature and enhance the effect of the VPD. II) Despite the site NAR shows the same response variation (slope) of other sites in the Southern Hemisphere, the response is shifted towards lower Fd values. This suggests that the standard irrigation treatment there might not be enough to ensure the adequate water supply.

Table 1.2 Outputs of linear regression model equal $\text{Log}_{10}(F_{rel}) \sim \text{Log}_{10}(\text{VPD} * \text{site} + (1 | \text{site}))$. CAM site was taken as a reference. * $P < 0.05$, *** $P < 0.001$.

Site	a	a _95%C.I.	a _Pr(> t)	b	b _95%C.I.	b _Pr(> t)
CAM	-1.517	-1.596; -1.436		1.174	1.092; 1.254	
CHIT	-1.128	-1.320; -0.935		0.867	0.671; 1.062	***
FRA	-1.206	-1.398; -1.013		0.901	0.704; 1.096	***
GEJ	-0.966	-1.159; -0.773		0.639	0.443; 0.835	***
NAR	-1.841	-2.033; -1.224	***	1.185	0.989; 1.380	
ORG	-1.459	-1.651; -1.266		1.123	0.927; 1.318	
SEB	-1.656	-1.848; -1.462		1.271	1.075; 1.466	

Seasonal impact on the response curves

Because the site has a significant impact in determining the transpiration response to VPD, sites were analyzed one by one. We selected months with the highest (June-November) and the lowest (August-February) soil water availability during the kernel formation period. The correlation between F_{drel} and VPD was always significant (Table 1.3). The intercepts of the linear regression on F_{drel} vs. VPD

resulted lower in all dry months (August and February) compared to the wettest months (June-November). In the Northern hemisphere, the average difference in the intercept between the wetter and drier months was 0.321. Interestingly, the site (ITA) with no irr had the lowest difference between wet and dry months (0.228), while the highest difference was in FRA (Figure 1.5).

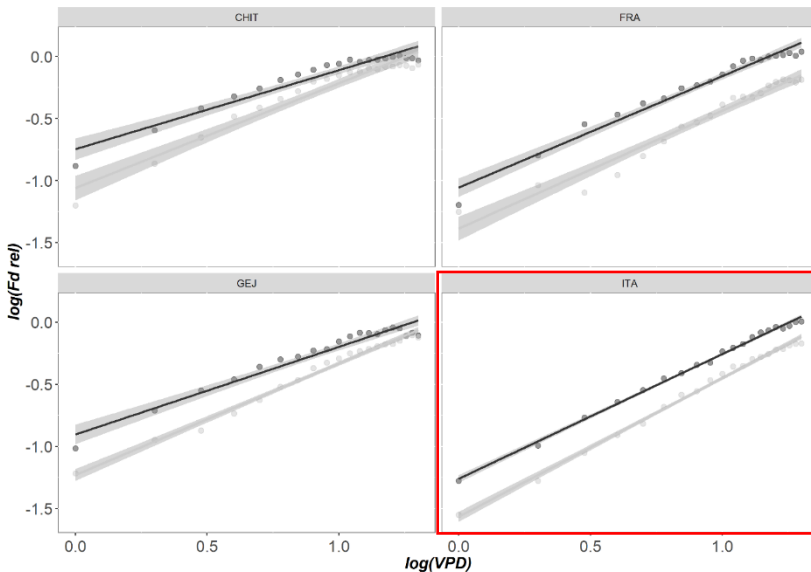


Figure 1.5 Relative sap flow (Fd_{rel}) vs. VPD in log-log scale in the four sites of the Northern Hemisphere. $VPD < 20\text{hPa}$. All sites are considered under standard irrigation treatment (std). ITA, which is the not irrigated site, is also included as a comparison term. Black markers refer to June (wet month), grey markers to August (dry month).

This suggests that the site ITA, precipitation are still enough to keep the transpiration condition relatively constant during the season, while in FRA, despite the irrigation was based on soil water monitoring, it did not ensure a constant water supply. However, in all sites with standard irrigation the slopes of the responses changed from the wet

to the dry months. Still ITA was the only to maintain the same slope of response in both periods.

*Table 1.3 Outputs of linear regression model equal $\text{Log}_{10}(\text{Frel}) \sim \text{Log}_{10}(\text{VPD}) * \text{month}$, for the months with higher (June) and lower (August) soil water content at four boreal sites. Treatment with no irrigation (ITA) is the only no irrigation site, while the others are under standard irrigation treatment. *P < 0.05, ***P < 0.001. All distributions of residuals resulted normal according to the Kolmogorov-Smirnov normality test.*

Site	Month	<i>a</i>	<i>a</i> _95%CI	Pr(> t)	<i>b</i>	<i>b</i> _95%CI	Pr(> t)
CHIT	June	-0.769	-0.873; -0.665	***	0.659	0.553; 0.766	***
	August	-1.083	-1.334; -0.831	***	0.857	0.601; 1.114	*
GEJ	June	-0.997	-1.070; -0.925	***	0.793	0.719; 0.866	***
	August	-1.323	-1.499; -1.149	***	0.984	0.805; 1.161	***
FRA	June	-0.984	-1.048; -0.919	***	0.81	0.754; 0.876	***
	August	-1.4	-1.555; -1.244	***	0.959	0.801; 1.118	**
ITA	June	-1.253	-1.286; -1.221	***	0.996	0.963; 1.029	***
	August	-1.481	1.560; -1.403	***	0.027	0.074; 1.103	

For what concerns the Southern hemisphere, we can notice a slightly different result (Figure 1.6). Still the correlation between *Fdrel* and VPD was significant (Table 1.4). The intercepts of the drier month is always lower compared to the wettest month, except the site ORG where no difference in intercept values was recorded. Thus, trees in ORG managed to transpire a comparable amount of water during the two extremes of the growing season. Indeed the difference in intercepts between February and November was 0.136 in ORG, while reached 0.548 in CAM, the highest difference between wet and dry month in all sites. Slopes in wet and dry month are significantly different in all sites, except NAR, where the trend of variation had the same slope in both months.

We found no significant difference in the response of *Fd* to VPD related to the cultivar (Tonda Gentile delle Langhe and Tonda di Giffoni).

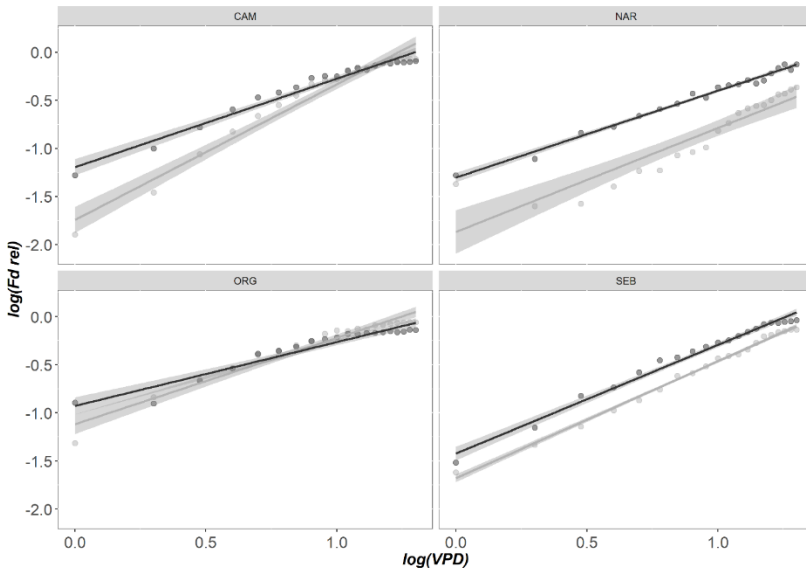


Figure 1.6 Relative sap flow (F_{drel}) and VPD in logarithmic scale in the four sites of the Southern Hemisphere. $VPD < 20bPa$. All sites are considered under standard irrigation treatment (*std*). Black markers refer to November (wet month), grey markers to February (dry month).

Table 1.4 Outputs of linear regression model equal $Log_{10}(F_{drel}) \sim Log_{10}(VPD) * month$, for the months with higher (November) and lower (February) soil water content at four austral sites. All sites are under standard irrigation treatment. February is used as a reference term. * $P < 0.05$, *** $P < 0.001$. All distributions of residuals resulted normal according to the Kolmogorov-Smirnov normality test.

Site	Month	<i>a</i>	<i>a</i> _95%C.I.	Pr(> t)	<i>b</i>	<i>b</i> _95%C.I.	Pr(> t)
CAM	November	-1.211	-1.484; -0.937	***	0.937	0.658; 1.126	***
	February	-1.759	-1.872; -1.645	***	1.425	1.309; 1.54	***
SEB	November	-1.413	-1.543; -1.283	***	1.119	0.987; 1.252	*
	February	-1.68	-1.733; -1.626	***	1.216	1.161; 1.271	***
ORG	November	-1.221	-1.469; -0.974	.	0.893	0.640; 1.145	*
	February	-1.357	-1.460; -1.255	***	1.088	0.983; 1.192	***
NAR	November	-1.382	-1.778; -0.986	***	0.969	0.556; 1.371	
	February	-1.834	-1.998; -1.67	***	1.046	0.879; 1.213	***

Treatment impact on in the response curves

For what concerns treatments we expected the same effect seen in the wet-dry months analysis: higher F_{drel} intercepts for treatments with increased water (std 2 and std+sp) and lower F_{drel} intercepts for treatments with less water (std 0.5). For this test, we selected only the sites of NAR, SEB, FRA and ORG during the drier month when the treatment effect should be more evident (Figure 1.7). Results show that in SEB, the double irrigation had a slightly higher intercept value respect to the standard (Table 1.5). In NAR the increased standard irrigation with sprinkler did not affect significantly neither the intercept nor the slope of the curves, suggesting that sprinklers did not have an effect on the sap flow increase. Slopes in NAR show that sprinklers did not affect the response of F_{drel} to VPD. Regarding treatment with decreased irrigation (std 0.5), in FRA we can notice that the decreased treatment had no significant impact in the pattern of F_{drel} with VPD respect to the standard. On the other hand, GEJ shows a significant difference between the standard and the std 0.5, which has a much lower intercept.

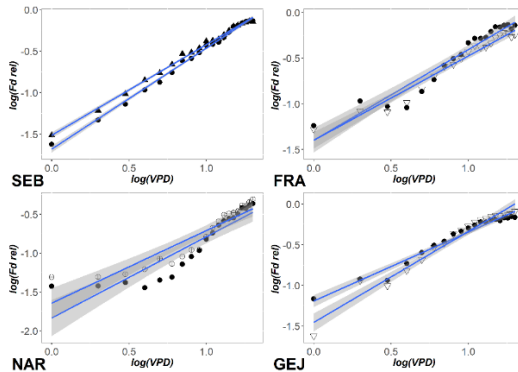


Figure 1.7 Relative sap flow (F_{drel}) and VPD in logarithmic scale in four sites during the driest month, $VPD < 20 \text{ hPa}$. Different irrigation treatments are shown with different markers: std = ●, std 2 = ▲, std +sp = ○, std 0.5 = ▽. All sites are considered under standard irrigation treatment (std). Black markers refer to November (wet month), grey markers to February (dry month).

Table 1.5 Outputs of linear regression model equal $\text{Log}_{10}(F_{rel}) \sim \text{Log}_{10}(VPD) * \text{treatment}$, for the sites where two treatments and evident impact on soil water content. On the left treatments with additional irrigation respect to the standard, on the right treatments with havened irrigation. * $P < 0.05$, *** $P < 0.001$. All distributions of residuals resulted normal according to the Kolmogorov-Smirnov normality test.

Additional irrigation treatments							
Site	Month	a	$a_{.95\%C.I.}$	$\text{Pr}(> t)$	b	$b_{.95\%C.I.}$	$\text{Pr}(> t)$
SEB	std	-1.68	1.716; -1.643	***	1.216	1.179; 1.254	***
	std 2	-1.514	-1.602; -1.426	***	1.092	1.003; 1.183	***
NAR	std	-1.835	-2.040; -1.629	***	1.046	0.837; 1.255	***
	std+sp	-1.643	2.1338; -1.147		0.934	0.429; 1.438	
Decreased irrigation treatments							
Site	Month	a	$a_{.95\%C.I.}$	$\text{Pr}(> t)$	b	$b_{.95\%C.I.}$	$\text{Pr}(> t)$
FRA	std	-1.4	-1.510; -1.29	***	0.995	0.883; 1.106	***
	std 0.5	-1.4	-1.665; -1.136		0.926	0.656; 1.194	
GEJ	std	-1.193	-1.283; -1.102	***	0.848	0.756; 0.94	***
	std 0.5	-1.456	1.674; -1.237	***	1.12	0.898; 1.342	***

Conclusions

The European hazelnut responded across all sites with a conservative strategy respect to the water status of the tree, with no difference respect to the cultivars involved: TGL and TG.

The relative value of sap flow helped in comparing different individuals at the site level and different patterns between sites.

In most of the sites where standard irrigation was in place, there was still a significant relative difference between the sap flow in the early growing season (June or November, higher transpiration) and the late growing season (August or February, lower transpiration). This shows that, despite the standard irrigation is running along the whole season, it is not sufficient to maintain transpiration rates constant at the same VPD during the important months for the kernel formation. This might be explained by the high evaporative demand during summer,

that persists at night and favors a higher stomata closure in the long term with consequent decrease in sap flow. At the same time, this might be the effect of the osmotic regulation occurring during the period of fruit sink activity (Badalotti et al., 2000). Indeed, Marsal et al. (1997) noticed a decrease in midsummer pre-dawn leaf water potential in both hazelnut and almond trees regardless the irrigation strategy (Reduced deficit irrigation, 100% and 130%). In particular Pisetta (2012) noticed that in July-August, despite higher soil water content, the leaf water potential decreased significantly respect to May (-1 MPa; -1.6 MPa), when soil water content was lower. This period corresponds to the kernel dry mass accumulation (Girona, 1994). From this long-duration experiment, we conclude that lower transpiration shall be always expected during the high sink period in hazelnut despite high water provision. Therefore, trees might not be able to withdraw additional irrigation in this period between July-August.

Interestingly, in the two sites where irrigation was not applied (ITA) or the wetting of the soil profile was difficult because of site-specific characteristics (very sandy soil at NAR), the slope of the curves remained unchanged during the season (almost parallel to each other). Indeed, the curves at these sites kept on diverging towards high VPD, while in other sites they converged towards 20hPa.

Overall, irrigation treatments had a little effect on transpiration. Double irrigation had a small effect on increasing the sap flow, while additional sprinklers did not have any effect respect to the standard irrigation. On half irrigation, which could be the best strategy for water saving at orchard level, we reported contrasting results according to the site. Thus, we can conclude that for a reduced irrigation strategy on hazelnut, more research might be needed after the works of Girona et al. (1994) and Marsal et al. (1997) to highlight the impact on nut yield and evaluate the water use productivity of such treatment.

CHAPTER 2
WATER REQUIREMENTS AND METHODS
FOR IRRIGATION MANAGEMENT
SUPPORT

CHAPTER 2.1

CALIBRATION OF GRANIER-TYPE (TDP) SAP FLOW PROBES

Pasqualotto, Carraro, Menardi, Anfodillo, 2019. Calibration of Granier-Type (TDP) Sap Flow Probes by a High Precision Electronic Potometer. *Sensors* 19, 2419. <https://doi.org/10.3390/s19102419>

Calibration of Granier-type (TDP) sap flow probes by a high precision electronic potometer

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Abstract

Thermal dissipation probe (TDP) method (Granier, 1985) is widely used to estimate tree transpiration (i.e. water evaporated from the leaves) because it is simple to build, easy to install and relatively inexpensive. However, the universality of the original calibration has been questioned and, in many cases, proved to be inaccurate. Thus, when the TDP is used in a new species, specific tests should be carried out. Our aim was to propose a new method for improving the accuracy of TDP on trees in the field. Small hazelnut trees (diameter at breast height 5 cm) were used for the experiment. The response of TDP sensors was compared with a reference water uptake measured with an electronic potometer system provided with a high precision liquid flow meter. We equipped three stems where we measured the sap flow density, the sapwood area (by using fuchsine), the total tree water uptake (reference), and the main meteorological parameters during summer 2018. Results confirmed that the original Granier's calibration underestimated the effective tree transpiration (relative error about -60%). We proposed a new equation for improving the measurement accuracy within an error of about 4%. The system proposed appeared an easier solution compared to potted trees and particularly suitable for orchards, thus contributing to improve the irrigation management worldwide.

Introduction

Methods for estimating the sap flow in trees are widely used in the ecological and agronomic context because the amount of water that flows through the stem is assumed equivalent to the water transpired by leaves. Compared to leaf-based measurements, all techniques based on sap flow provide continuous measurements that can be automatically collected and up-scaled to the whole stand (Čermák et al., 2004; Smith and Allen, 1996). Several methodologies to measure sap flow have been proposed, such as the heat pulse velocity (Marshall, 1958), the thermal dissipation proposed by Granier (Granier, 1985) and the heat field deformation (Nadezhdina et al., 2012). Among these, the thermal dissipation method (TDP) is the most common, mainly because probes are easy to install and to build, they require low energy supply and are relatively inexpensive (Braun and Schmid, 1999). Also it was proved that with self-made sensors the required budget is further reduced, still achieving good performance compared to the commercial sensors (Davis et al., 2012). For all these reasons, TDP was widely tested in forest stands as well as in orchard contexts. Especially in productive orchards, the main aim was to find the best tradeoff between irrigation input and yield, involving fruit trees as almond (Fuentes et al., 2013), mango (Lu and Chacko, 1998), peach (González-Altozano et al., 2008), olive (Charfi Masmoudi et al., 2011) and apple. Indeed, the sap flow monitoring can be used for irrigation design based on plant necessities (González-Altozano et al., 2008). However, while a species-specific calibration is essential to obtain a reliable outcome of sap flow and quantify the water uptake in tree plantations, still TDPs are rarely calibrated for the specific species.

Granier (Granier, 1985) performed an empirical calibration for the TDP system thus deriving sap flow density (F_d) as:

$$F_d = a k^{-b} \quad (1)$$

In this equation $k = (\Delta T_0 - \Delta T) / \Delta T$, where ΔT is the temperature difference between the two probes (the heated vs. the reference one), ΔT_0 is the maximum temperature difference (i.e. the condition corresponding to zero flow) and $b = 1.231$. Depending on the constant a , the unit that defines Fd can assume several dimensions (i.e. for $a = 4.284$, $\text{dm}^3 \text{ dm}^{-2} \text{ h}^{-1}$). Although the original calibration experiment (Granier, 1985) involved few species (*Pseudotsuga menziesii* (Mirb.), *Pinus nigra* (Arnold) and *Quercus pendunculata* (Ehrh.)), Granier et al. (Granier et al., 1990) asserted that the TDP technique was species independent. Indeed, for some diffuse wood species, the Granier's outcome was generally confirmed (Braun and Schmid, 1999; Bush et al., 2010; Chu et al., 2009; Clearwater et al., 1999; Do and Rocheteau, 2002; Ford et al., 2004; Gutiérrez and Santiago, 2006; Isarangkool Na Ayutthaya et al., 2010). However, in many other cases, standard Granier's equation of the TDP system resulted inaccurate. Many studies reported a relevant underestimation of the real sap flow density (Bovard et al., 2005; Chan and Bowling, 2017; Clearwater et al., 1999; de Oliveira Reis et al., 2006; Lundblad et al., 2003; Mei et al., 2018; Niu et al., 2015; Peters et al., 2018; Silva et al., 2008; Smith and Allen, 1996; Steppe et al., 2010; Taneda and Sperry, 2008; Tatarinov et al., 2005; Wullschleger et al., 2011; Xie and Wan, 2018). The causes might be related to anatomical (e.g. heterogeneity of vessels distribution), physiological (e.g. variation of radial profile of sap flow), technical (e.g., sensor features and installation) or other methodological aspects (e.g., calibration set-up). For all these reasons, probe calibration must be performed before an extensive use as suggested by Smith and Allen (Smith and Allen, 1996) and Steppe et al. (Steppe et al., 2010).

A number of studies exist about different calibration methods for TDPs. A simple method consists in comparing the TDP records with the difference in weight loss of potted plants or shoots (Lu and Chacko, 1998; McCulloh et al., 2007; Van Leperen and Madery, 1994). This method is probably the easiest to implement. However, especially on

large potted trees where the total mass reaches up to 700kg (sum of soil, pot, roots and all above ground mass), the relative mass variation due to evapotranspiration is minimal (from 10 to 50g) (McCulloh et al., 2007) and large errors can be introduced even with a precise balance. Alternatively, the test on TDPs occurs with the gravimetric method, i.e. the application of a volume of water that is forced to flow into cut wood segments by positive pressure (de Oliveira Reis et al., 2006; Granier, 1985; Steppe et al., 2010). The application of sub-atmospheric pressure to small wood segments was also used as a calibration option (Bush et al., 2010; Fuchs et al., 2017). Still, all cut-stem method with either positive or negative pressure, create a highly artificial setting that is far from the real condition of a tree by removing all the leaves and partitioning the stem tissues. Because the sap flow moves along a gradient of negative pressure from the soil to the atmosphere that is leaves-driven, it would be ideal to test the system in a context that is as close as possible to the field conditions without segmenting the hydraulic path as in the cut-stem. The “whole tree” potometric system, i.e. the whole above ground part of the tree, responds to these requirements. The potometric system was widely used with herbaceous plants or twigs on which semi-automated records can be collected (McDonald et al., 1981; Sheriff, 1972; Van Leperen and Madery, 1994). The potometer for TDP calibration was adapted for whole aboveground trees in broadleaves and conifers few times (Knight et al., 1981; Sun et al., 2012). However, in these studies the variation of water volume to be compared with TDPs remained at a low record frequency (every 30 minutes or even not mentioned). Often, the water up-take by trees is measured by the water needed to restore a predefined value, which does not provide any information on the flow rate dynamic. Low accuracy of records remains an issue in field experiment where visual quantification of water loss from tanks was more widely used. Indeed, high-resolution balances remain expansive devices and not easy to use for field experiments.

This work aims at performing an accurate calibration test for Granier's TDP in the field on a tree species of commercial interest, maintaining the calibration setting as close as possible to the natural hydraulic transport conditions. For the calibration, we installed a whole-tree potometric system connected with a high precision flow meter that provided continuous measures at the same time with TDPs. As a test species, we selected the hazelnut (*Corylus avellana* L.), a diffuse-porous species, which is greatly expanding in cultivated area because the fruits are very requested both as healthy food and in confectionery industry. We hypothesize that, it is possible to provide a correction factor for the original Granier's equation to provide a more accurate estimation of tree transpiration. This new relationship might be easily used for managing more precisely the irrigation scheduling and thus achieving an optimization water resources.

Materials and Methods

2.1 Study area

The study was carried out on two mature individuals of *Corylus avellana* L. growing in the countryside (site A) and in a mountain woodland area (site B) of Veneto region (NE Italy), respectively in the province of Treviso (45°53'04"N, 12°05'27"E) and Belluno (46°24'05"N, 12°21'58"E). Both sites are located in areas where the species regenerates naturally at an altitude of 150 and 650 m a.s.l. Trees showed their natural multi-stem habit: about 3-4 m tall and with 8 to 10 stems at the base. The first individual was part of a natural hedgerow, while the second was included in a mixed stand of maple (*Acer pseudoplatanus* L.), ash (*Fraxinus excelsior* L.) and beech (*Fagus sylvatica* L.). The climatic conditions between the sites differ mainly in terms of temperatures, with annual averages between 12.2 and 7.6 °C in the lower and higher altitude respectively. The annual precipitations is slightly more abundant in site A (1038 mm year⁻¹) compared to site B (952 mm year⁻¹).

The experimental test took place during summer 2018 in order to take advantage of the highest VPD, which is the main driver for sap flow. The test was repeated three times on three different stems in the two sites. The first test occurred on site A at the end of June (day 177) on a stem with a diameter of 55mm and 4.5 m in height. We repeated the test on site B in mid-July (day 198) and at the end of August (day 240), on two stems selected from a second plant (diameters 54 and 52mm, 4.5 and 4.3m tall, respectively).

2.2 Experimental set up

2.2.1 The Granier's TDP

On each stem, we installed two TDPs (20 mm-long) in opposite positions. The Granier's system consists of two needle-shaped probes (originally 20 mm-long), both incorporating a fine wire T-type thermocouple. Each thermocouple sensing tip is placed in the middle of the probe length. Externally, the probes are provided with a coil winding of an insulated resistance constantan wire (copper–nickel alloy). Despite in many works this distance was changed (Sun et al., 2012; Wullschleger et al., 2011) we decided to keep it at 10cm to avoid underestimation of sap flow (Iida and Tanaka, 2010). Before to insert the sensors in the stem, their windings are inserted in a metal sleeve shaped as an aluminum micro-tube, 2 mm in diameter (Lu et al., 2000) that makes the thermal exchange with the wood more uniform. When the system is operative, the upper probe is permanently heated (power 0.2 W) with a constant current. The energy due to the Joule effect is dissipated by convection and conduction at heated probe surface, in relation to the sap flow amount. The temperature difference (ΔT) between the probes, reaches the highest value when the sap flow (F_d) is zero and progressively decreases when the water uptake increases. The sensors were installed at a distance of 10 cm to avoid possible mutual thermal interference. For stems with relative small diameter, we can consider that the two sampling points might represent the variability of flow around the stem circumference with minimum

alteration of the conductive area. Indeed, the azimuthal variation of sap flow variation is, in general, measured by using two (Delzon et al., 2004; Nourtier et al., 2011) to four probes (Shinohara et al., 2013; Tsuruta et al., 2010) in relation to stem diameter. In small stems (diameter < 8-10 cm) as in our case, two probes in opposite sides (i.e. North and South aspect) are enough (Daum, 1967; Fichtner and Schulze, 1990; Oren et al., 1999) thus limiting the error below 10% (Chiu et al., 2016). Moreover, we placed the probes relatively near to the living crown, where the azimuthal variability is even reduced (Köstner et al., 1998; Loustau et al., 1998)

2.2.2 Potometer and liquid flow meter

The response of TDP sensors was simultaneously compared with the reference sap flow representing the effective stem water uptake. The reference sap flow was measured applying the potometric technique using an electronic system (Figure 2.1). The monitored stem was excised at the base and immersed in a deionized water reservoir 5 liters capacity (cross section of 3.5 dm²). The reservoir was connected with a plastic pipe (Figure 2.1-3) to a second jug. Both water reservoirs were placed on a flat platform and covered with a plastic film to prevent the superficial evaporation.

According to the communicating vessels principle, the depletion in volume of water (Vt) in the first jug, caused by the transpiration activity, produces an equal variation of water level (dh) in both reservoirs. The variation of water volume (dV_1) in the first jug promotes a flow (dV_2) that moves through the connection pipe from the second jug. Therefore:

$$Vt = dV_1 + dV_2 = dhA_{w1} + dhA_{w2}$$

(2)

Where A_{w1} and A_{w2} are the cross-sections of jugs 1 and 2, respectively. The relation between the transpired water Vt and the flow dV_2 , that is measured with the flow meter, is:

$$Vt = dV_2 \left(\frac{A_{w1}}{A_{w2}} + 1 \right)$$

(3)

We used two identical jugs with the same basal area A_w , but the jug 1 has a lower water volume because of the stem. Hence, taking into account the stem cross-sectional area S_a , the Equation 3 turns into:

$$Vt = dV_2 \left(\frac{A_w - S_a}{A_w} + 1 \right) = dV_2 \frac{2A_w - S_a}{A_w}$$

(4)

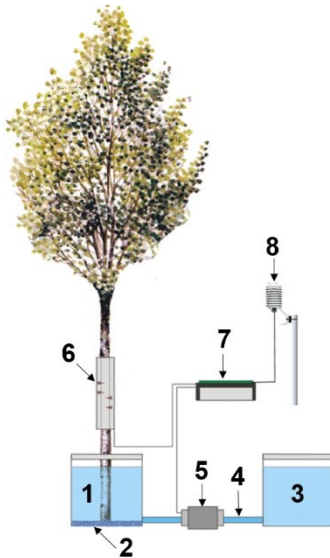


Figure 2.1. Experimental setup. 1) Potometer with the first water reservoir; 2) Porous layer; 3) Second water reservoir; 4) Connection pipe; 5) High precision flow meter sensor; 6) Sap flow sensors; 7) Data acquisition system; 8) temperature and relative humidity sensor.

To measure the real-time variation of water flow, we installed in the connecting pipeline a high precision liquid flow meter (Figure 1(5), Sensirion, mod. SLQ-QT500). This sensor covers the flow range between -120 ml min^{-1} and $+120 \text{ ml min}^{-1}$, has a fast response time down to 50ms and high sensitivity and accuracy down to the lowest flow (Figure 2.2).

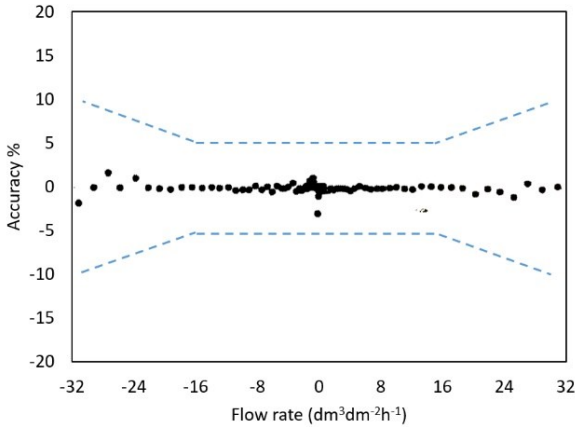


Figure 2.2 The accuracy expressed in percent is reported with the flow rate unit ($\text{dm}^3 \text{dm}^{-2} \text{h}^{-1}$) for the sap flow meter Sensirion, mod. SLQ-QT500. The accuracy test on the sensor is provided by the producer of the sensor, while the figure and the scale were adapted to our case study.

It is designed to monitor flows in alcohols, solvents, oil, fuel or adhesives and it is highly recommended to measure fluids with high viscosity. However, because of its micro-thermal measures and large inner diameter, the measurement performance does not depend on the viscosity itself. Indeed it is particularly suitable where small changes in flows must be detected such as in large diameter pipes (Øvstebø, 2016) or xylem sap movements which flow velocity is estimated to be on average 4 mm s^{-1} (Taiz and Zeiger, 2002). The sensor has a very low power consumption (104 mW, current drain 6 mA) and can be connected via USB cable to PC for the real-time monitoring and data collection with a dedicated software. Alternatively, it can be connected to a datalogger. In this case, an analog cable interface provides an output voltage with 12 bit resolution that corresponds to the measured flow rate. By default, the analog output is set to 5 V at no-flow, 0 V for negative max flow and 10 V for positive max flow. Whether the output voltage exceeds the input range of the datalogger, a voltage divider (whether homemade or commercial, i.e. VDIV 10.1 Campbell Sci.) could easily solve the problem.

The connection to the SLQ-QT500 is established by directly connecting to the sensor 1/4" outer diameter (OD) Teflon™ tubing. A variety of suitable fittings, connectors, and unions is available from different suppliers. Most connectors for 1/4" OD plastic tubing will work. The exact type is determined by the user depending on the specific application. The flow meter has been robustly engineered to ensure the internal Teflon™ tubing to a Quartz interface. This provides a dependable seal without using any additional adhesives or gasket sealing materials. This tightly integrated design prevents any disassembly without damaging internal components.

In all trials, the potometric measurements occurred few days after the installation of TDP sensors in order to verify that the TDP response was steady and regular. Before to cut the stem the mother plant was irrigated during the night to obtain a full hydration. The selected stem was also tided with ropes to the surrounding stems to keep it stable after the cutting procedure. We excised the stem before sunrise when the sap flow is negligible in order to reduce the risk of vessels cavitation. The stem cut occurred in few seconds using a manual saw, then the stem was quickly transferred into the water reservoir. The potometer system was installed right close to the stump to preserve the original position of the stem. This allowed also for an easy self-support of the sample, which crown wedged in the rest of the living branches. The experimental protocol was repeated on each of the three stems.

2.2.3 Meteorological parameters

In addition to the sap flow, we also measured the air temperature and the relative humidity using a digital relative humidity and temperature probe (Campbell sci., mod. CS215). The sensors were installed right above the tree crown to get the real atmospheric conditions. A summary of these parameters is reported in Table 2.1.

Table 2.1. Environmental conditions during the sampling days (177: 26 June; 198: 17 July; 240: 28 August) in summer 2018. Precipitation is the sum of all events during the season. Minimum, mean and maximum daily temperature (T°C), relative humidity (RH%) and vapor pressure deficit of the atmosphere (VPD).

day	T °C			RH %			VPD hPa		
	min	mean	max	min	mean	max	min	mean	max
177	14.10	20.18	25.60	46.00	71.56	85.50	0.75	7.87	17.31
198	12.83	18.12	23.94	54.82	80.18	97.80	0.46	4.71	13.43
240	10.70	16.86	23.43	48.16	76.71	96.10	0.53	5.58	14.93

All the employed sensors were connected to a data acquisition system (Datalogger Campbell sci. mod. CR1000) with a recording frequency of 30 seconds. The data storage frequency was of 5 minutes computed as the average of 12 values.

2.2.4 Model fitting and daily sap flow calculation

We derived from the TDP row data the k value of Equation (1). This value was compared with the output of the real sap flow density from the liquid flow meter in order to find the corrected values of coefficients a and b of Equation (1). To provide a representative model for all the samples we followed the approach that assumes as the best fitting model curve that with the lowest root mean square error (RMSE) and the highest Willmott index of agreement (D) between the corrected and the reference sap flow. D is a standardized measure of the degree of model prediction error and it is defined by Equation (5):

$$D = 1 - \frac{\sum_{i=1}^n (X_i - P_i)^2}{\sum_{i=1}^n (|X_i - \bar{Y}| + |Y_i - \bar{Y}|)^2} \quad (5)$$

It describes the relative co-variability of X and Y about an estimate of the 'true' mean \bar{Y} . A value of 1 indicates a perfect match, and 0 indicates no agreement at all (Willmott, 1981).

The daily sap flow calculation was used to compare in the three days the real water uptake from the potometer, the original Granier's equation (F_d) and the corrected equation (F_d). Thus, the mean daily

value of these sap flow densities ($\text{dm}^3 \text{dm}^{-2} \text{h}^{-1}$) has been multiplied by the sap wood area (dm^2) and by 24 (h).

2.2.5 Conductive sapwood area

To estimate the sapwood area during the experiment we perfused the stem with a 0.05% aqueous solution of Acid Fuchsine (CAS 3244-88-0), on the base of the protocol described by Sano et al. (Sano et al., 2005). At the end of every experimental session, the stem was sectioned at the insertion of the heated probe of the TDP and the section was scanned. Finally, the sapwood area was measured analyzing the images with the freeware ImageJ software (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA). In order to have comparable measurements with the TDP system, we scaled the potometer data (i.e. the total stem water uptake) to the sap flow density dividing them by the sapwood area. We assumed that the sap flow density was homogeneous around the circumference: the assumption should be realistic due to the relatively small size of the measured stems.

Results

3.1 Comparing TDP outputs and liquid flow meter

Our tests were performed during days with different atmospheric evaporative demand for testing the calibration in a wide range of conditions (i.e. from zero to $2 \text{ dm}^3 \text{dm}^{-2} \text{h}^{-1}$). (Figure 2.3 and 2.4).

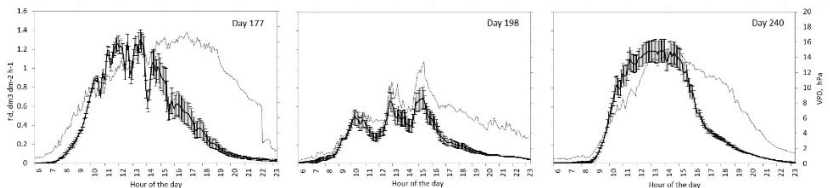


Figure 2.3. Average sap flow densities from two probes per tree in day 177, 198 and 240 of summer 2018. Bars represent the standard error. VPD (hPa) appears in background.

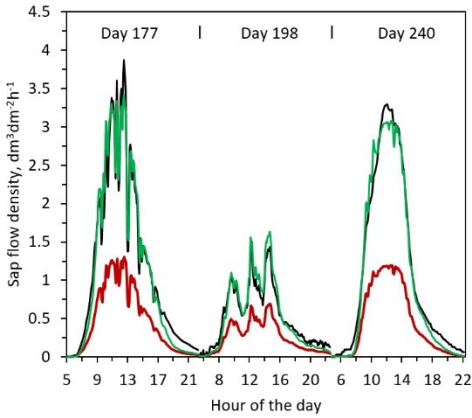


Figure 2.4. Reference sap flow density (black line), original TDP output, Granier's equation (red line), corrected TDP output, corrected equation (green line) and VPD (grey line), for the three performed tests.

TDP output (both from original Granier's equation and corrected) exhibited a well synchronized response with the reference sap flow, i.e. the measurements of water uptake derived by the flow meter (Figure 2.4). The best synchronization between TDP outputs and reference flow is optimized within a time lag < 5 min ($r^2 = 0.991$, see also Table 2.3), while it worsen gradually with lags > 5 (e.g. $r^2=0.975$, lag > 5 min; $r^2=0.952$, lag > 10 min).

When the standard factors a and the exponent b proposed by the Granier's calibration were used, the underestimation of the sap flow density measured with the TDP clearly emerged from the deviation from the linear correlation between the volume of water derived by the potometer and the term k of Equation (1) (Figure 2.5). The sap flow density estimated using Granier's equation (yellow line) is correct only when $k < 0.2$. For higher values of k , the factors a and b caused an underestimation compared to the real water flow in all sampled trees.

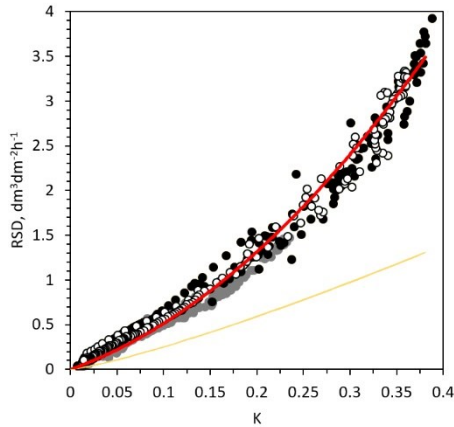


Figure 2.5. Relationship between k (Eq. 1) and reference sap flow density (RSD; black dots: day 177; grey dots: day 198; white dots: day 240). The red line is the species-specific calibrated model (Eq. 5), the yellow line represents the original Granier calibration.

Considering the data of all trees together, we derived a fitting curve to estimate the sap flow density (Figure 2.5, red line) modifying the original coefficients a and b of Granier's Equation (1).

Thus, we obtained the following fitting equation for the corrected sap flow density (Fd_c):

$$Fd_c = 13.86 k^{1.45} \quad (6)$$

The performance of the new calibration is summarized in Table 2.2 and 2.3 where main statistics and daily total relative errors on sap estimation are reported. Statistical indicators in Table 2.2. confirmed the goodness of the fitting between estimated and reference sap flow density from the potometer. Root mean square error (RMSE) and mean absolute error (MAE) were relatively small, while coefficient of determination (r^2) and Willmott index of agreement (D) were very high and the slope of regression was almost 1 (0.995). Figure 2.6 shows the relationship between the reference sap flow density and both the sap flows calculated with the Granier's equation and the corrected one.

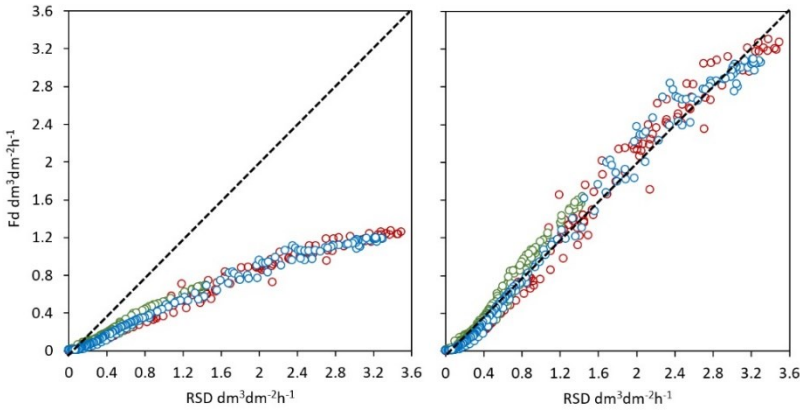


Figure 2.6. Relationship between the reference sap-flow density (RSD, from liquid flow meter) and the sap flow calculated with the Granier's equation (F_d , left) and the new corrected equation (F_{d_c} , right). Different colors show different stems at day 177 (red), 198 (green), 240 (blue).

Table 2.2. Statistical indicators of Granier's (F_d) and model performance (F_{d_c}) as compared to the reference sap flow density, in terms of root of the mean square error (RMSE), mean absolute error (MAE), coefficient of determination r^2 , Willmott index of agreement (D) and slope of regression (m).

F_d equation	N	RMSE	MAE	r^2	D	m
F_d	623	0.064	0.54	0.986	0.705	0.403
F_{d_c}	623	0.13	0.1	0.991	0.995	0.995

Table 2.3. Daily sap flow and relative errors of corrected original equation.

	Day 177	Day 198	Day 240
Measured liters by flow meter	6.63	2.82	5.77
Estimated liters by Granier's equation	2.43	1.3	2.30
Estimated liters by corrected equation	6.37	2.88	5.52
Relative error of Granier's equation (ϵ %)	-63.36	-54.04	-60.14
Relative error of corrected equation (ϵ %)	-3.87	1.89	-4.29

In respect to the total daily sap flow (Table 2.3), the potometer accounted 6.63, 2.82 and 5.77 liters respectively on day 177, 198 and 240, while the corrected equation estimated 6.37, 2.88 and 5.52 liters.

Granier's equation exhibited a substantial relative error (ϵ %), underestimating more than 60 % of total transpiration in the days with the highest VPD and about the 54 % in the day (198) with less evaporative demand. The corrected equation improved the estimation, with a little underestimation (around -4 %) on days with higher VPD (days 177 and 240) and a little overestimation (1.89 %) in occasions of lower transpiration (day 198).

The performance of the model is graphically reported in Figure 2.4. The reference (black line) and the corrected (green line) sap flow, except for some few high peaks of sap flow density, overlap quite well with the application of the new coefficients. Comparing the new corrected output with the original TDP (red line) we notice an evident improvement.

3.2 Estimation of the sapwood area

In all the three stems, the entire wood section was colored by the perfused fuchsine transported with the sap flow to the leaves. Along the radial profile, rings show only little differences in coloration intensity (Figure 2.7). However, since the TDP covered the entire radial length, we are confident that the sensors averaged out this small variability.

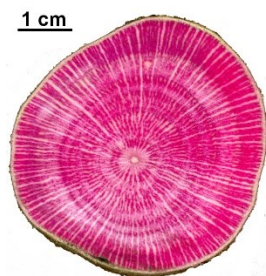


Figure 2.7. Transversal section of one of the stem perfused with a 0.05% aqueous solution of Acid Fuchsine. The whole area appeared active and around the 25% was constituted of parenchymatic rays (white stripes).

Discussion

We have described the application of a high precision liquid flow meter for low flows applied to a whole-tree potometer in order to calibrate TDP. This allowed us to propose a new species-specific correction factor for hazelnut, which increases markedly the reliability of the TDP for this species. The calibration can be easily applied for any species of interest.

We developed a model based on the corrected Granier's equation by manipulating the coefficients a and b (Equation 1). The model resulted to have a satisfactory performance because the sap flow density estimation error was very low (<4%). Nevertheless, the corrected equation can be considered appropriate for temperate regions. Indeed, we validated the model in a range of values of k values (Equation (5)) not higher than 0.4, i.e. for a maximum sap flow density of about $2 \text{ dm}^3\text{dm}^{-2}\text{h}^{-1}$. In warmer/drier climates where this threshold might be overcome, the model reliability could not be tested because of the mean tree size of samples and the relatively low vapor pressure deficit of the atmosphere in our sites. For these reason, such calibration would need to be improved for higher k values.

The potometer applied to the whole aboveground biomass is probably the one that mostly respects the natural conditions of the tree hydraulics, conserving the leaf area and the stem hydraulic path intact with respect of the real micro-meteorological factors. This technique removes possible sources of errors common to the cut stem calibration method that may induce lower TDP records when positive or negative pressure are applied (McCulloh et al., 2007). At the same time, the use of the liquid flow meter was the most precise back up measure of transpiration in terms of record frequency and water volume quantification. The liquid flow meter revealed to be very sensitive to external disturbances (i.e. vibration induced by wind). Nevertheless, we obtained a stable signal that did not present spikes or undefined oscillations, which might be explained by the fact that our sample trees

were growing in the understory of the forest in semi-protected environment. Additionally, the sensor proved to be highly user friendly in terms of electronic interfacing and hydraulic installation. No leakages have been reported in the joints. Still, in the potometric approach some limitations exist. In general, the negative pressure driven by atmospheric conditions can not be controlled as in the laboratory, unless a large climatic chamber is predisposed. As in many other calibration methods with cut stems, the risk of cavitation of xylematic vessels during the cutting phase remains an unknown bias despite we handled it by cutting the base of the stem for 15 more cm in order to exclude possible damaged tissues. Using the whole-tree potometer, the water source was applied to the entire wood section.

Despite the TDP system showed a good response time compared to the reference, also in this species the original Granier's equation for the TDP confirms to be not fully reliable. Our experiment confirmed that also in *C. avellana*, as in many other broadleaves, the TDP method produces an underestimation of the sap flow density from 54 to 63% respect to the reference sap flow measured with the potometer. These results are completely coherent with the observation of Steppe et al. (2010), who also tested the TDP on other broadleaves. Indeed, the degree of underestimation observed in their study was a TDP underestimation of 60% compared to the gravimetric method on trunk segments of *Fagus grandifolia* (Ehrh.). Fuchs et al. (Fuchs et al., 2017) found a lower underestimation of TDP, about 40%, obtained with the calibration on cut stem with negative pressure. Differences in the underestimation esteems may lie in the different calibration methodologies that use either positive or negative pressure to generate artificial flow in cut-stem calibrations. Still Fuchs et al. (2017) suggest some causes for the underestimation of TDP related to calibration methods: leakages (positive pressure) or soaked water (negative pressure) can respectively decrease TDP temperature and increase the insulation of tissues around the TDP (wounded tissues or cavitation that decrease thermal dissipation). The effect is to induce artificially

lowered sap flow recorded by probes. However, the cause for the underestimation of TDP is still not entirely disclosed, while the importance of defining the actual conductive sap wood surface remains a crucial issue to make the TDP output comparable to the real water uptake. The wood anatomy of hazelnut may have played an important role in the effectiveness of the TDP system. Indeed, about 25% of the sap wood area resulted to be not conductive due to an abundant presence of wide (0.5-1 mm) parenchyma rays (Figure 2.7). Furthermore, because hazelnut is classified as a semi-ring-porous to diffuse-porous species (Crivellaro and Schweingruber, 2013), the wood anatomy presents a scattered distribution of vessels on the transversal section. This might be the reason behind the original TDP calibration failure (Clearwater et al., 1999) in which the equation fails when part of the probe is in contact with non-conductive xylem. Indeed, these anatomical characteristics of hazelnut explain why the coloration with dye resulted to be homogeneous, but at the same time, we noticed some differences in the records of the two TDPs on the same stem (Figure 2.4). Indeed, some TDPs might have partially intercepted a parenchyma ray. Despite that, the averaged measures of TDP records on each stem remained well correlated with the reference flow showing to represent the general trend of the section (Figure 2.4).

The main cause for the general underestimation of TDPs may be related to the uneven radial profile of sap flow, i.e. the steeper the radial velocity gradient of sap flow, the higher the underestimation of the probes (Clearwater et al., 1999). The differences in the radial conductivity of the xylem depends on many factors: wood anatomy (Berdanier et al., 2016) cavitation and senescence (Tyree and Zimmermann, 2002) as well as differences in canopy conductivity (Hernandez-Santana et al., 2016). Therefore, to improve the quality of TDP measure a species-specific characterization on sap flow radial profile shall be performed for hazelnut.

Conclusions

We can conclude that the calibration with the potometer applied to the whole aboveground tree mass and the liquid flow meter is a valid method to adjust the TDP equation and it can be applied on most tree species with small (4.5 cm to fit the TDP minimum length) to medium diameters (20-25 cm). The only limitation for tree size remains the possibility to secure the tree standing during the cut and the calibration period. The system is also closer to the natural condition of growth compared to cut stem method. Furthermore, compared to calibration with potted trees on a balance, this system avoids the preparation of pots with many years in advance for the experiment and can be applied with a portable system in the forest without the support of a lab nearby. This allows to maintain the sampled trees in their original growing condition. In this way, a corrective equation for TDP could be used for a variety of tree species in orchards where a precise quantification of water needs is required. In these contexts, a species specific and accurate calibration remains a paramount step to improve plantation management. For this purpose, the potometer equipped with the liquid flow meter shows to be a promising method to achieve reliable water consumption predictions and limit excess irrigation in the agronomic sector.

CHAPTER 2.2
QUANTIFICATION OF THE TRANSPIRATION IN
EUROPEAN HAZELNUT ORCHARDS

Introduction

Predicting tree water use in orchards is challenging because transpiration depends on a large number of biological and environmental variables. Thus, soil water and meteorological parameters should be carefully monitored (temporally and spatially) as well as the physiological responses of trees. At the same time, there is an increasing need to understand how the agriculture sector can improve water management for food production to increase water use efficiency and productivity. Indeed, in all world countries the water footprint related to agricultural production covers the largest share of the single country impact (Hoekstra and Mekonnen, 2012). In addition, water availability for crops is predicted to decrease severely due to global warming and population increase (Jiménez Cisneros et al., 2014). At the same time, high precision water use monitoring is not of easy access for many growers and irrigation managers, who need to convert research outcomes into operative irrigation scheduling. Thus, the crop coefficient remains the most used method to estimate the water supply to recover transpiration losses. However, a more flexible and tree based strategy to predict transpiration in orchards has been proposed. For instance, the transpiration ratio method, defined as the actual daily water use divided by the potential water use in trees grown under field capacity was reported to be a reliable and easy method to quantify and schedule the irrigations at orchard level (Fernández et al., 2008). Moreover, Pereira et al. (2006) showed that a leaf area based transpiration estimate is correct and easy to understand for orchards. Still, they highlight that the choice of reference well-irrigated trees, must be careful not to fall into extensive over-irrigation issues. On the contrary, under-irrigated trees shall be avoided for species that have no benefits from reduced irrigation strategies. The European hazelnut is very sensitive to water deficit (Bignami et al., 2011, 2009). Water saving strategies, such as the regulated deficit irrigation showed to affect negatively the performance of this species (Girona et al., 1994; Marsal

et al., 1997) compared to almond (Girona et al., 2005; Romero et al., 2004) or grapes (Acevedo-Opazo et al., 2010) for which this treatment is even beneficial for the fruit quality. Therefore, in the nut production, irrigation is paramount especially outside its natural ecosystem or under a climate change perspective. Despite that, no precise knowledge on its optimal water requirements is defined for the species at the whole tree level. This leads growers to organize the irrigation scheduling on an as-needed basis calibrated on local experience. In this study, we considered the tree-based transpiration derived from sap flow measurements in eight world-distributed hazelnut orchards. A long-term data series together with biometric data served to estimate the leaf area transpiration at each site and during the growing season. The same dataset served to propose a model approach that describes the potential water use of the species expressed as daily liters per unit of leaf area. The transpiration is simply related to the mean daily VPD of the atmosphere and can be up-scaled to the plantation level according to the specific parameters of the orchard. Thus, the transpiration volume can be estimated on the average site conditions of VPD.

Material and methods

Biometry of sampled trees

The mean basal area across sites was of 1.70 dm² (s.d. 0.8) and was calculated as the sum of all sprouts belonging to the same mother tree, when trees grew in the multi-stem habit. The basal area derives from diameters sampled at 10 cm from the ground (in order to consider all secondary branches). The bark thickness was about 2 mm at all sites. The mean crown volume calculated from the projected area was of 28.66 m³ (s.d. 2.1). The tree leaf area LA (m²) was estimated from the allometric equation $LA = 0.16 * D^{2.22}$ ($R^2 = 0.93$) where D is the diameter (in cm) at the base of each stem. The relationship was established by using both cultivars TGL by Pisetta (2012) in Baldissero d'Alba and TG in Georgia (2016, data not published). Total tree leaf area is the

cumulated leaf area of all sprouts belonging to that tree. Values ranged between 14 and 99 m² of leaf area per tree, where the minimum values corresponds to Australian trees (more details in the Chapter “Study Areas”, Table II)

Data processing

For this study, sap flow data included 7 different world-distributed hazelnut orchards during three growing seasons from 2016 to 2019. The total transpired water per day was derived from the single branch sap flow density Fd_c multiplied by the whole tree cross sectional area swa (sum of cross sectional area of several branches, dm²). The *ad hoc* calibration for Granier’s thermal dissipation probes for hazelnut (Chapter 2.1) was applied to calculate the sap flow density (Fd_c). The leaf transpiration density (l m⁻² of LA) on a daily basis is expressed as = $Fd_c \cdot swa / LA \cdot 24$. The up-scale to the plantation is possible through the knowledge of plantation density.

For the model, we only considered trees treated with the standard irrigation treatment (n.3 trees per site). Thus, the daily leaf transpiration was obtained per each sample at each site. The daily mean leaf transpiration and the daily mean VPD (classes of 0.1 hPa) were aggregated per day and site.

Results and discussion

Orchard and leaf transpiration

The transpiration ET at the orchard level resulted very different across sites ranging between 0.79 and 7.73 mm day⁻¹ average water loss (Figure 2.8). This is explained by either the very low soil water availability in some areas (e.g. site NAR with very sandy soil or ITA, which is the only rain fed) and by the different plantation densities or tree sizes in the orchards. CHIT and FRA resulted the sites with the highest water loss.

Water requirements and methods for irrigation management support

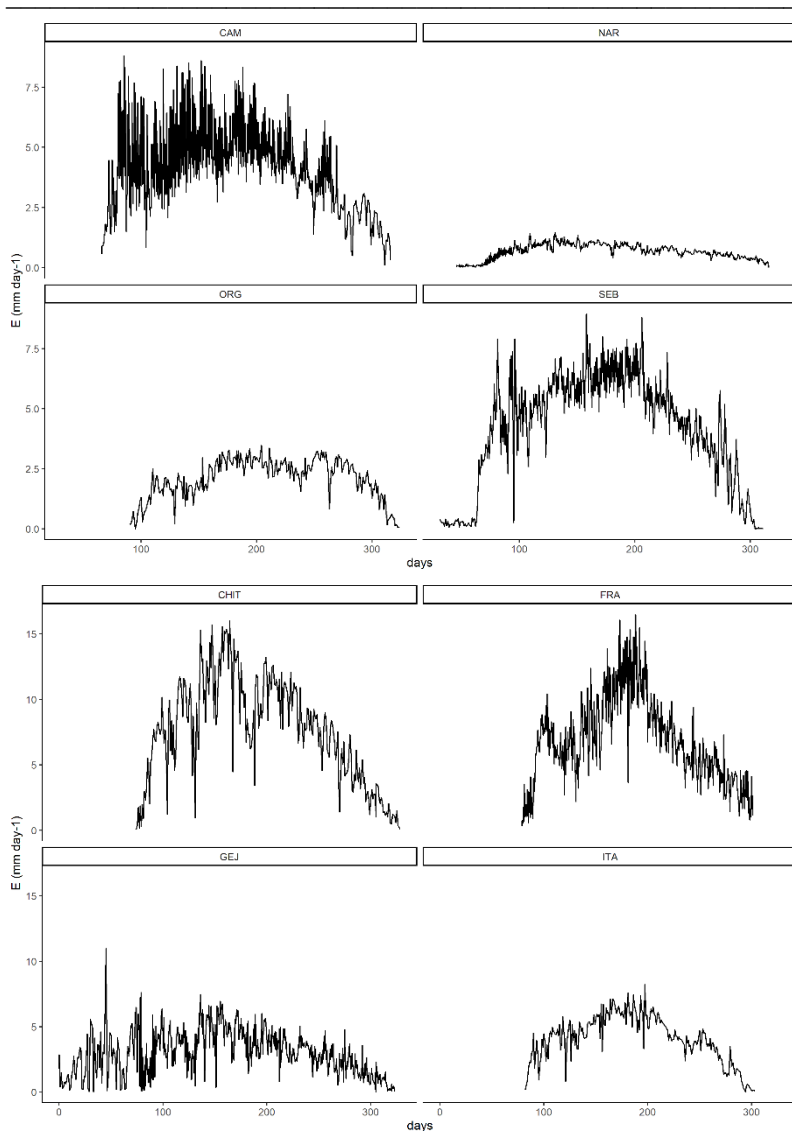


Figure 2.8 Evaporation from canopies in mm per day along the growing season in the Southern (top) and Northern Hemisphere (bottom). Daily means of 3 years data collection. Days refer to the days of the year for Northern sites, while for the Southern sites they have been converted to Boreal days of the year to allow for the comparison.

Indeed, there the total tree leaf area is about 90 m² per tree and the density is relatively high (667 trees per hectare). At these sites, the estimated LAI resulted about 6 (Table 2.4). However, when the transpiration is reported to the leaf area unit, it is more uniform across sites, suggesting that the leaf functionality is a quite constant trait.

The daily leaf transpiration across the growing season in all irrigated sites (Figure 2.9) shows a variation between 1.02 and 2.55 l m⁻² day⁻¹. The monthly variation highlighted some variability about the beginning of the transpiration activity. For example in NAR (Australia) the transpiration season starts with one month in advance compared to ORG, which is still located on NSW Australia, but in a different climatic region. June is the month when the leaf transpiration culminates in Northern sites, while the highest values are reached in January in the Southern sites (Figure 2.10). Only NAR shows an anticipation of the peak of two-months.

Sites with higher leaf transpiration in June/November (group *a*) had well coupled crowns with the atmosphere together with high soil water content. This might have lead to higher transpiration compared to orchards with greater tree leaf area or scarce soil water. Indeed, the orchard density and the tree size did not allow the crowns to close, thus the whole leaf area was exposed to similar atmospheric condition (VPD, radiation) which act as drivers for the transpiration. The available soil water content ensured a full transpiration of the entire crown. On the contrary, in NAR where small, highly coupled trees had scarce soil water availability, the leaf transpiration was at its minimum for the species. In FRA the combination of highly uncoupled crowns (tall, dense trees) with not fully accessible water resources lead to a similar result.

Water requirements and methods for irrigation management support

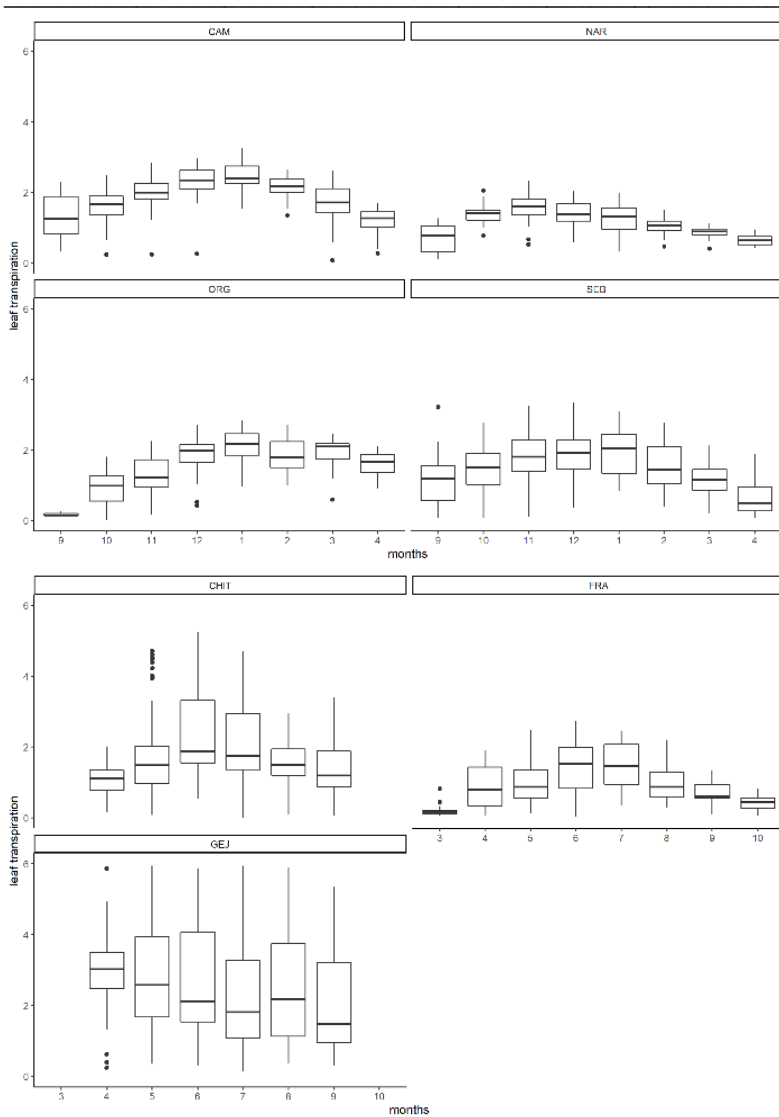


Figure 2.9 Daily leaf transpiration ($l\ m^{-2}\ LA\ day^{-1}$) per month in 7 sites with standard irrigation. Southern Hemisphere on the left, Northern Hemisphere to the right.

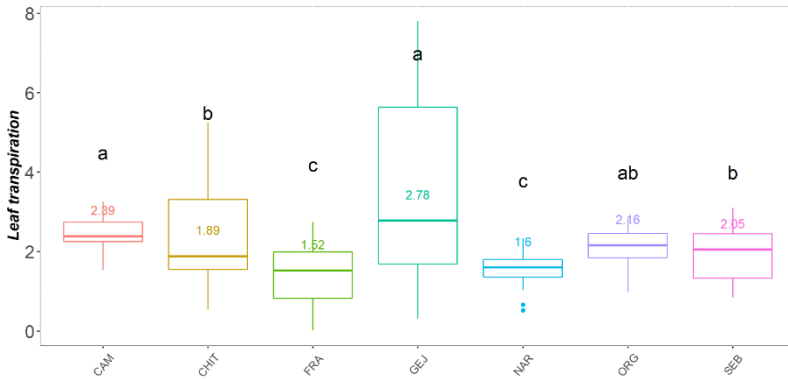


Figure 2.10 Values of daily leaf transpiration of the month with highest transpiration in the growing season ($l m^{-2} day^{-1}$). Boxplots corresponds to sites, letters on top of the boxes report the result of the non-parametric post-hoc test of Dunn, Bonferroni method. The median value is reported for each site.

The leaf transpiration of an average growing season in hazelnut was up scaled to the plantation level using the tree density (Table 2.4). Biometric data helped in deriving the mean tree leaf area per site. The cumulated transpiration refers to the actual water consumption of trees under the existing irrigation management and climatic conditions (evaporative demand of the atmosphere and precipitations). A further implication of these results concerns the possibility to estimate the tree water consumption of an orchard while leaf area increases. For instance, CHIT and SEB had the same plantation density and a comparable leaf transpiration, while CHIT has a higher tree leaf area. In this case, we can estimate the seasonal water consumption in SEB depending on the crown surface increase with age or in CHIT when pruning occurs to reduce the tree leaf area.

Table 2.4 Plantation density and average leaf area per tree and hectare, leaf area index, leaf transpiration during the season expressed as daily mean and cumulated per growing season. The cumulated values is also expressed as liters per leaf area unit per day and total Mega liters per season. The growing season is considered from October to March in Southern Hemisphere and from May to September in the Northern Hemisphere.

Site	Trees ha ⁻¹	Tree LA m ²	LAI	mm day ⁻¹	mm season ⁻¹	l m ⁻² LA day ⁻¹	Ml ha ⁻¹ season ⁻¹
CAM	333	54	1.8	4.28	779.6	2.38	7.80
CHIT	667	90	6.0	7.53	1371.0	1.80	13.71
FRA	667	91	6.1	6.13	1115.9	1.20	11.16
GEJ	667	21	1.4	2.82	513.5	2.40	5.14
ITA	400	77	3.1	3.99	725.4	1.54	7.25
NAR	500	14	0.7	0.79	143.0	1.12	1.43
ORG	500	27	1.4	2.24	407.8	1.66	4.08
SEB	417	73	3.0	5.19	945.1	1.70	9.45

A simple model to recover transpiration losses

All models that predict the responses of living organisms are subjected to a trade-off between accuracy, which requires to measure and use all important variables, and the practical application of the model, which requires to use only few easy-to-measure variables. To propose a model for orchards irrigation that needs many variables is unrealistic when the aim is to address growers. Indeed growers are in general poorly equipped in terms of meteorological stations, soil water measurement or sap flow sensors. This convinced us to propose a very simple model based only on one variable (VPD), with the hope that such strategy might help growers to start to use such simple tool.

Three reference curves are proposed to guide the estimation of the leaf transpiration based on mean temperatures and relative humidity.

The mean daily leaf transpiration is estimated from daily mean VPD by mean as $y=0.6661*\ln(x) + 0.2915$ (Figure 2.11, red line), $R^2=0.78$. The logarithmic form of the curve is used to include most of the filed observations. This prediction allows to reintegrate the transpiration losses based on the previous day mean VPD. This curve is modelled on data of temperate areas, where maximum daily VPD is below 20hPa.

In these temperate conditions, trees can reach a maximum transpiration up to $3 \text{ l m}^{-2}\text{day}^{-2}$ when VPD= 25 hPa (Figure 2.11, top black line).

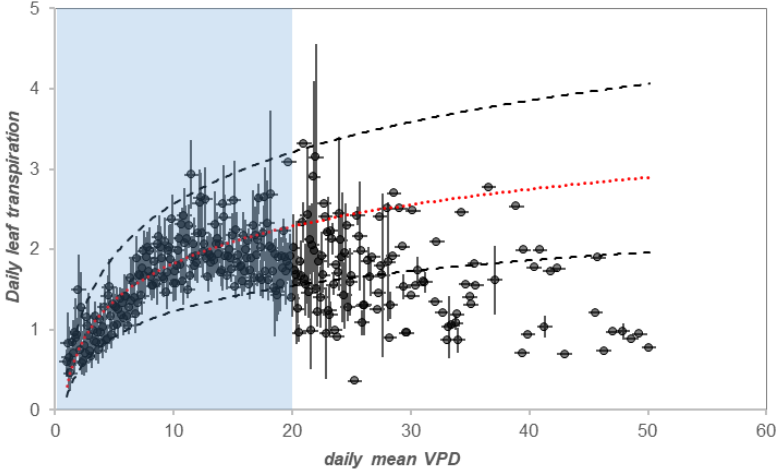


Figure 2.11 Mean daily leaf transpiration ($\text{l m}^{-2} \text{ day}^{-1}$) and mean daily vapor pressure deficit (hPa) in 7 irrigated orchards (CAM, SEB, CHIT, GEJ, FRA, NAR, ORG). The top black dashed fits the maximum values per unit of VPD class: $y = 0.9291 \cdot \ln(x) + 0.4269$. The dashed red line corresponds to the means (data $< 20 \text{ hPa}$): $y = 0.6661 \cdot \ln(x) + 0.2915$, $R^2 = 0.78$. The bottom dashed black line corresponds to the minimum values: $y = 0.4581 \cdot \ln(x) + 0.1724$. Error bars are \pm standard errors. The shaded area is the range of VPD in temperate areas.

As we can see from Figure 2.10, in general we have a quicker response of daily leaf transpiration to daily mean VPD until 10 hPa (see also Pasqualotto et al., 2018). This is always the most critical interval to keep monitored. Above the top black curve, all additional irrigation shall be considered lost, as trees showed not to transpire from experimental data. The minimum daily leaf transpiration is taken as a reference not to overpass in temperate regions (Figure 2.10, bottom black curve). Still the same curves can be used (shifted to the bottom) for more extreme climates. For instance in Australian sites, where the maximum average daily VPD is above 20hPa, the red curve will represent the maximum

potential transpiration, while the bottom black curve will be the reference for the average transpiration.

According to the measurements collected in irrigated orchards, the maximum value of actual leaf transpiration fitted by the curve occurred between 20-25 hPa. A decline in transpiration rates was observed above this value, even with high irrigation support. This suggests that a variety of triggering factors are likely to interfere with the transpiration process at daily mean VPD > 20 hPa. In Australian orchards, VPD daily means are around 18 hPa and reach peaks between 39-52 hPa, high temperatures and low relative humidity occur often with intense solar radiation. In these areas, the leaf transpiration occurrences below the potential of temperate areas can be explained by the severe damage to the photosynthetic tissues (leaf burning has been reported extensively on leaves).

Conclusions

Results on leaf transpiration collected in seven irrigated hazelnut orchards with different plantation densities and tree leaf areas, indicate that on a daily time scale the transpiration ranges between 1 and 2.5 l m⁻²day⁻¹, that was similar to values found in walnut and apple orchards (Pereira et al., 2006). This would confirm that the leaf area based transpiration has low variability across orchards trees and canopy sizes. In addition, the technique to measure tree leaf area with the allometric relation for hazelnut lead to plausible results of transpiration. Because this technique is based on a simple equation and on the sampling of sprout diameters, it can be performed with a basic training of even non-highly qualified staff. The coupling/decoupling effect of trees from the atmosphere caused by crown size and tree density affected the daily leaf transpiration. The month with the highest transpiration was the third after the leaves emergence (Solar and Stampar, 2011) and corresponds to ovary and ovule growth (Beyhan and Marangoz, 2007).

Therefore, the application of an optimal water supply in this period is paramount.

The daily leaf transpiration includes also nocturnal water loss, which fraction was not analyzed separately in this study. Still, nocturnal evaporation has sometimes being reported as an important component of the total water balance in orchards (Escalona et al., 2013; Fuentes et al., 2013; Medrano et al., 2015; Pereira et al., 2006), and it might be a focus for future steps on this topic.

The model derived by these data estimates the water volume per unit of leaf area, which needs to be reintegrated at the orchard level from simple meteorological parameters. A set of three curves allow to adapt the model according to the orchard climatic setting (temperate or warm). Despite the crop coefficient for hazelnut has been suggested by Gispert et al. (2005), this approach shall avoid the need of such indicator and provide and updated effective tool for growers.

CHAPTER 3
TREE TRANSPIRATION AND POTENTIAL
PRODUCTIVITY: LINKS AND LIMITATIONS

CHAPTER 3.1
LIMITATIONS OF CANOPY CONDUCTANCE
EFFICIENCY

Introduction

Photosynthesis is the source of energy and biomass, which allow biological processes, but how the products of carbon fixation are allocated among different plant tissues and in reproduction is still a central issue (Enquist et al., 1999). This is even a more intriguing question in trees of low-stature communities where regeneration occurs through clonal growth and seed dispersal at the same time (Reekie and Bazzaz, 2000). According to a multiple species study on plants (Niklas and Enquist, 2002), the annual reproductive biomass would scale allometrically as the 0.8-power of the vegetative biomass. Thus, suggesting that the scaling of the biomass devoted to reproductive allocation is similar in both angiosperms and gymnosperms (Niklas and Enquist, 2002), but increases less than proportionally respect to the aboveground biomass. Few studies considered the allometric scaling of reproductive tissues (Niklas and Enquist, 2003; Thomas, 1996), but rarely they involve orchard species (Brym, 2016). Indeed, the high level of manipulation of vegetative tissues through pruning and sprouts removal causes alteration of natural allometric scaling (Brym and Ernest, 2018).

Still, to predict the amount of reproductive biomass in orchard would be extremely important to improve their management. In hazelnut orchards, this is not an easy task, because of the alternate bearing of reproductive events.

However, according to the allometric scaling laws, the vegetative and reproductive biomass should be always dependent on the efficiency of carbon assimilation. In this sense, also the potential for fruit production in orchard should be, ultimately, a fraction of the total carbon uptake. Because carbon exchange occurs through the stomata, and a measure of the degree of stomata aperture is the leaf conductance g (Jones, 1992), the latter can be a proxy for assessing the total carbon uptake, partially devoted to reproduction.

At the tree level, the conductance G_t refers to the capacity of gas exchange (water vapour out flow and CO_2 inflow) of the whole tree crown, which includes a wide variety of heterogeneous leaf conditions (Medrano et al., 2015).

In stands where the decoupling coefficient is low (Ω , Jarvis and McNaughton, 1986), stomata experience a VPD similar to the free atmosphere, and the total conductance of the canopy is assumed to be driven largely by stomata aperture (Chapin III et al., 2002; Hogg and Hurdle, 1997; Köstner et al., 1992). Thus, canopy conductance G_t can be estimated from the rate of xylem sap flow density (Fd) and air vapor pressure deficit (VPD) on a tree basis: $G_t = Fd/VPD$ (Arneeth et al., 1996; Köstner et al., 1992). G_t includes two components: a stomatal component (average of different canopy leaves) and an aerodynamic component, which is function of the orchard structure.

Thus, when it is considered on a relatively long-term scale (weeks, months), G_t can be a proxy for carbon uptake of the tree, which in turn should determine also the carbon pool for reproductive allocation.

In this study, we explored the simple relationship between VPD and canopy conductance G_t as a contribution to understand the limitations of hazelnut potential reproductive biomass in orchards and we tested the influence of irrigation treatments on G_t vs. VPD. Further, we conducted a simple experiment for clarifying which sources of carbon are used for nut production: if only those assimilated by the leaves of the same branch or/and resources already stored within the stem tissue and mobilized towards the expanding nuts (Chapter 3.2).

Materials and Methods

Estimation of total tree canopy conductance

The total water vapor transfer capacity or conductance (G_t) is estimated from the raw xylem sap flow density Fd and the air vapor pressure deficit (VPD) on a tree basis (Köstner *et al.*, 1996). Diurnal sap flows and VPD were extracted from the dataset described in Chapter 1, which includes eight study areas. The initial data set was subset to obtain diurnal data from 6 AM to 6 PM for the growing season: May-August for the Northern hemisphere, November-February for the Southern hemisphere. The relative G_t is calculated as $G_{rel} = G_t/G_{t90^\circ}$. G_{t90° is the value at the 90th quantile of the full series of G_t . Relative

values helped in comparing different sites and highlight the intervals of VPD in which the canopy conductance is maximized. *Grel* is calculated on a single-tree basis in order to normalize for possible differences between individuals. The frequency of *Grel* classes as well as VPD classes has been considered, in order to understand the likelihood of maximization of crown gas exchange at each site. The effect of different irrigation treatments was analyzed separately in each study area with a non-parametric two-sided test. All statistical analysis and visual representations were conducted using the software RStudio version 3.5.1 (2018).

Results and Discussion

The daily pattern of relative canopy conductance showed some differences between sites (Figure 3.1). During the day, the maximum relative conductance was reached at 10 AM, except in NAR, where it occurred at 8 AM. Four sites (CAM, SEB, ORG and ITA) showed a pronounced morning peak of *Grel* followed by a similar sudden decrease before noon. All three of them had a standard irrigation while ITA was the only rain fed. The rest of the sites had a less rapid increase of conductance in the morning and preserved a quite constant conductance during the rest of the day. The maximum *Grel* experienced per VPD unit during the growing season also changed between sites (Figure 3.2).

CAM, with *Grel* max=0.79, was the site that was closer to its maximum potential *Grel*, while FRA was the site with the lowest, *Grel* max=0.57, indicating that conditions of maximum gas exchange are rarely met. The maximum *Grel* during the growing season was always below 1, thus very far from the potential *Grel* registered in these sites. This shows that the absolute maximum conductance experienced by trees occurs outside the four months considered important for the kernel development. On the other hand, the canopy conductance almost never reached relative values below 0.20. In other words, tree crowns

never reduce the gas exchange to zero (between 6AM and 6PM), even at very high VPD values. This phenomenon can be either generated by stomata that do not close completely, or by a relatively high cuticular conductance derived from lack of leaf wax (Dawson et al., 2007), which might prevent transpiration at high VPDs.

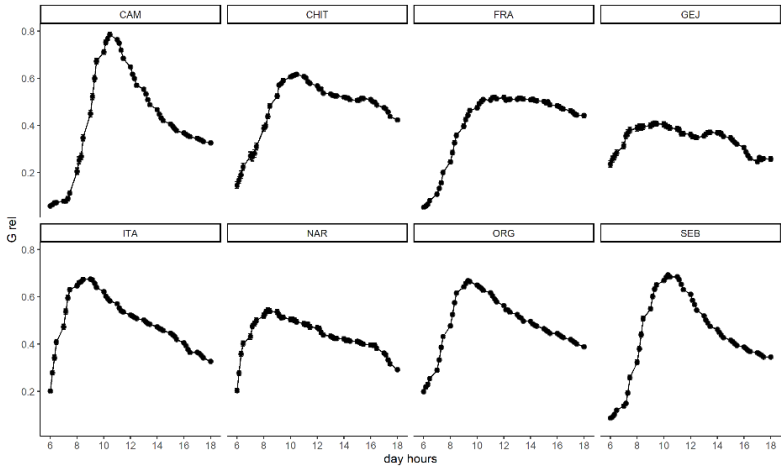


Figure 3.1 Pattern of average relative canopy conductance G_{rel} during the day hours in eight sites during 4 months of the growing season between 6 AM and 6 PM. All sites are irrigated with a standard irrigation, except ITA which is rainfed. Error bar refer to the standard error.

The maximum G_{rel} was recorded between 7 and 17 hPa across sites (Table 3.1). The interval of VPD that includes G_{rel} values $>80\%$ respect to maximum was between 10 and 12 hPa units, distributed normally around the peak of G_{rel} . However, FRA, NAR and ORG had a wider interval of high G_{rel} occurrences around the maximum respect to the other study areas. The diversity of window width between sites might be due to slight differences in plantation densities, soil properties, orchard exposition and pruning technique which shapes the canopies differently (see the chapter on Study areas).

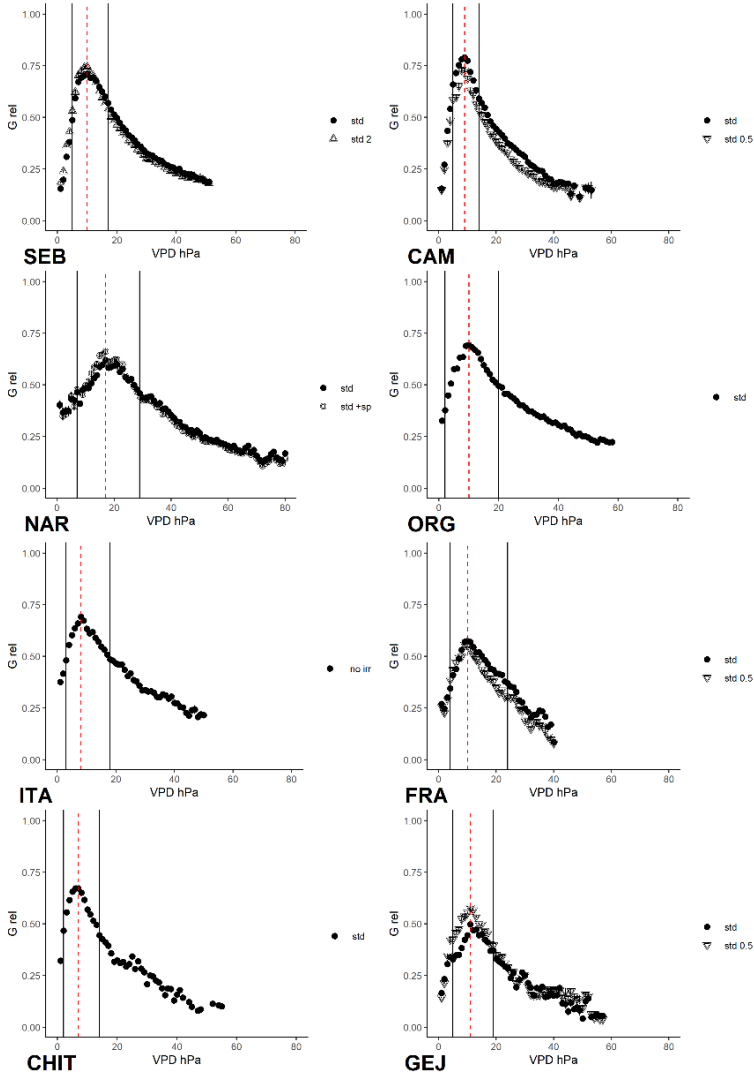


Figure 3.2 Patterns of relative canopy conductance (G_{rel}) with VPD classes (hPa). Vertical lines show the values of VPD at which $\max G_{rel}$ occurs in different sites (dashed red line) and at which we can expect a decrease in G_{rel} of 20% from the maximum (black solid lines). Labels at the bottom-left of each figure indicate the specific site ID. Treatments at each sites are shown in the legend at the right of each box.

Tree transpiration and potential productivity

Table 3.1 Maximum *Grel* during the growing season (May to August for Northern Hemisphere, November to February for the austral) and corresponding VPD. VPD interval in which *Grel* decreases of less than 20%

Site	<i>Grel</i> max	VPD (hPa) at <i>Grel</i> max	Min and max VPD (hPa) at <i>Grel</i> max -20%
SEB	0.74	10	5 - 17
CAM	0.79	9	6 - 14
GEJ	0.58	11	8 - 19
CHIT	0.67	7	5 - 14
FRA	0.57	10	4 - 24
ITA	0.69	8	3 - 18
NAR	0.66	17	7 - 29
ORG	0.69	10	4 - 20

The medians of diurnal VPD occurrences during the growing season was examined in the sites (Figure 3.3). In most of them, the *Grel* “high potential productivity window”, i.e. when *Grel* remains above 80% of its maximum, occurs around the median of VPD occurrences at the site. In few cases (SEB, CAM, ITA and NAR), this window is just slightly lower respect to the median.

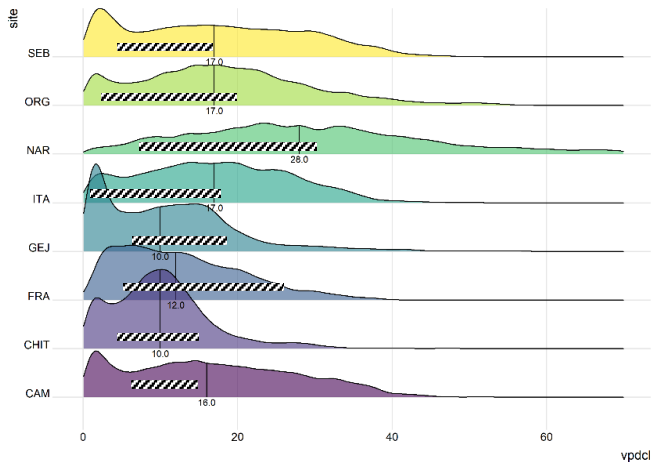


Figure 3.3 VPD frequencies in diurnal datasets (6 AM to 6 PM) in all sites. Medians are shown as vertical black lines with the specific value. Black striped bars show the window of VPD values in which *Grel* maximization occurs, i.e. when *Grel* remains at the 80% of its maximum.

Chapter 3

This suggests that in these sites we might expect a sensible reduction of productivity in case of an increase of VPD as predicted by global circulation models. Indeed, trees would remain for most of the time in a non-optimal condition, which might lead in the long term to reduced carbon uptake and potential productivity.

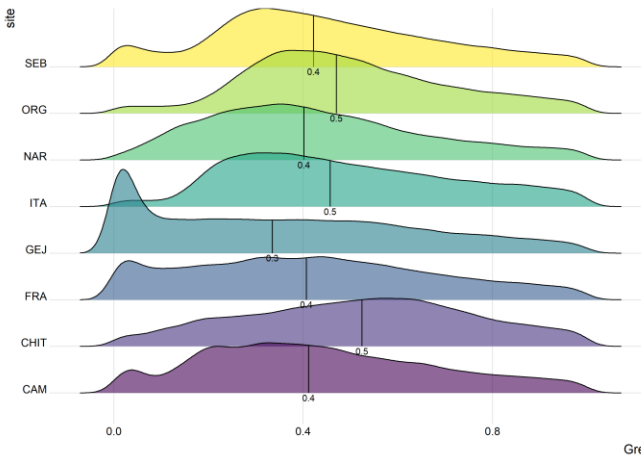


Figure 3.4 *Grel* frequencies in diurnal datasets (6AM to 6PM) in all sites. Medians are shown as vertical black lines with the specific value.

CHIT, ORG and ITA resulted to be the sites that most frequently experienced high *Grel* (Figure 3.4). In other words, they are most frequently closer to an optimal conductance and more prone to fix higher quantities of CO₂. The others might need interventions to shift the actual median of *Grel* towards higher values.

The irrigation effect on *Grel* resulted not significantly different between the standard irrigation treatment and both additional (std 2, std+sp) and halved (std 0.5) irrigation strategies (Table 3.2 - Figure 3.5). This result, if confirmed by other measurements, would be particularly important because it shows that the behavior of the species in term of

VPD responses is relatively rigid and not easy to modify. This might open some options for irrigation strategies aimed at saving water with only small effects on productivity.

Table 3.2 Unpaired two sample Wilcoxon test in 5 sites where two treatments of irrigation are in place during the growing season. Medians of respective treatments are reported with their p-values. Significance in brackets.

Site	treatments	Medians <i>Grel</i>	p-value (sign.)
CAM	std – std 0.5	0.33 - 0.26	0.24 ()
GEJ	std – std 0.5	0.21 - 0.22	0.37 ()
FRA	std – std 0.5	0.36 - 0.31	0.19 ()
NAR	std – std+sp	0.28 – 0.26	0.67 ()
SAB	std – std 2	0.34 - 0.33	0.78 ()

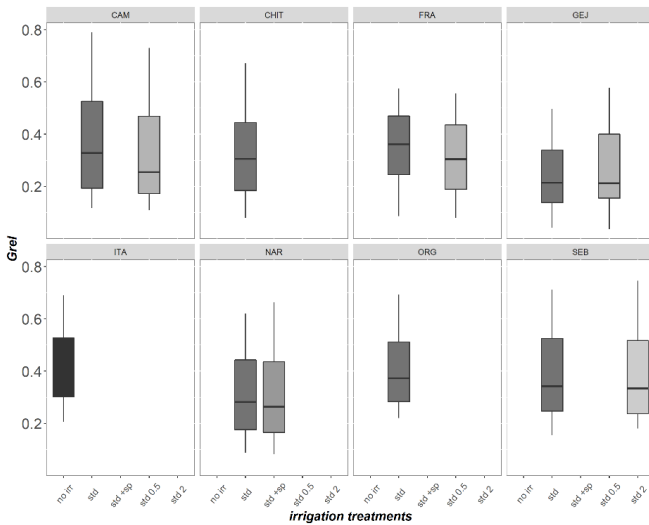


Figure 3.5 *Grel* in the eight study areas during the growing season. Treatments are reported with boxes of different colour intensity from black (no irr) to light grey (std 2)

Conclusions

The analysis of canopy conductance on 7 irrigated and one rain fed hazelnut orchard showed that trees spend most of the central months of

the growing season in a non-optimal condition respect to the potential conductance of the species. On the other hand, the minimum conductance experienced across sites never approaches zero values at high VPD (>40 hPa), suggesting that the control over the reduction of canopy conductance during extreme events of dry-hot atmosphere is not sufficient to avoid water loss. This trait was observed both in irrigated and rain-fed sites. Tardieu and Simonneau (1998) actually found that the effect of VPD on stomatal control can cease in well-irrigated field-grown plants. In a review on minimum leaf conductance Duursma et al. (2019) shows numerous examples of irrigated plants that, compared to water stressed treatments, have a higher minimum conductance. Their observation together with these findings rise some concerns about the efficiency of highly irrigated orchards. Indeed, most of the water distributed on the ground would lead trees to be more exposed to water loss at extreme VPD. On the other hand, it might help plants to cope with sudden heat waves (Drake et al., 2018). We could observe that on the total occurrences of diurnal growing season conductance the median is between 0.4-0.5 *Grel*. Orchard management should act towards the shift of *Grel* median towards higher values, either decreasing occurrences of low *Grel* or increasing the high values. In general neither doubled irrigation nor half irrigation had effects in modifying the median of *Grel* occurring at the monitored sites. However, we identified site dependent windows of VPD in which *Grel* is maximized. We expect that the most is the orchard structured to favor these VPD conditions, the higher will be the carbon stocked in trees available for nut production. Indeed canopy structure variation may largely influence the water use efficiency of orchard (Cohen and Fuchs, 1987; Cohen and Naor, 2002). In this sense ground irrigation might not be the main strategy to increase canopy conductance, especially in these areas where high VPD are frequently high.

CHAPTER 3.2
MANIPULATING C SOURCES & SINKS AND EFFECTS
ON NUT PRODUCTION

Pasqualotto, G., Carraro, V., De Gregorio, T., Huerta, E.S., Anfodillo, T., 2019. Girdling of fruit-bearing branches of *Corylus avellana* reduces seed mass while defoliation does not. *Sci. Hortic. (Amsterdam)*. 255, 37–43.
<https://doi.org/10.1016/j.scienta.2019.05.016>

Girdling of fruit-bearing branches of *Corylus avellana* (L.) reduces the seed mass while defoliation does not.

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Abstract

Hazelnut (*Corylus avellana* L.) is an important nut crop with clear mast seeding behaviour leading to alternate yield and, thus, to management issues. It was hypothesized that masting is associated to accumulation/depletion of carbon within storage tissues (i.e. stem, large branches, roots). We hypothesized that fruit yield depends both on carbon fixed by leaves on the same branch and carbon stored in the main stem. We therefore conducted a manipulation of carbon sink-source resources on fruit-bearing branches of *C. avellana* cv. ‘Tonda di Giffoni’ by combining girdling and defoliation on a set of branches. We measured the number and diameter of nuts and leaves, the dry weight of fruits and seeds and the amount of blanks over the growing season. We also estimated the whole tree leaf area by allometric equations and the tree yield by collecting all nuts separately on 15 trees at maturity. The average seed dry mass in the un-defoliated girdled treatment was significantly lower than the control (0.5 vs. 0.9 g) suggesting that the fruit-bearing branches need to import carbon from the main stem and that the carbon autonomy of fruit-bearing branches is not supported during kernel maturation process. We also observed that the correlation at whole tree level between leaf area and seed mass is strong in non-masting years, while it becomes weaker in masting years reinforcing the hypothesis that the productivity of masting yields also relies on stored resources.

Introduction

Hazelnut (*Corylus avellana* L.), is one of the major nut crops in the world (Boccacci and Botta, 2009) but its cultivation still remains at an early stage of domestication compared to other similar orchard trees (e.g. almond, walnut). Hazelnuts are often managed in their natural multi-stem habit (Fideghelli and De Salvador, 2009), while world production is still based on the selection from wild types. Breeding program efforts have only recently been empowered and are mainly to strengthen the resistance to possible pathogens (Leadbetter et al., 2015; Mehlenbacher, 2009; Muehlbauer et al., 2018). One of the greatest management issues in hazelnut is the pronounced mast seeding behavior that causes a twofold variation of nut yield in the following years.

The fruit production on trees is the result of a trade-off by the plant between vegetative growth and reproduction (El-Kassaby and Barclay, 1992; Obeso, 2002, 1993). This phenomenon is particularly pronounced in species that show a mast-seeding behavior where years of massive and synchronous production of seeds alternate with one or more years of negligible production (Janzen, 1971; Kelly, 1994; Kelly and Sork, 2002).

The resource accumulation (or budget) hypothesis (Crone and Rapp, 2014) explains seasons of mast seeding as a phenomenon that severely weighs on carbon reserves stored in different plant tissues in order to support the production of fruits. This process requires the plant to accumulate enough reserves in the following seasons to recover its stores (Kelly and Sork, 2002).

Instead, the theory of branch autonomy suggests that foliated branches are carbon autonomous for the most of the growing season (Sprugel et al., 1991). In other words, the majority of carbon sources for fruit production would derive from photoassimilates fixed in the same fruit-bearing branch or in the nearest leaves, and does not depend on stored reserves (Hasegawa et al., 2003). Indeed, each branch condenses the main tree growth functions: carbohydrate formation, wood growth,

sugar and starch storage, and reproduction (Sterck et al., 2005). Even high carbon demanding infructescences might be supplied by photosynthetic bracts preserving the idea of carbon autonomy of fruiting branches (Aschan et al., 2005).

Hoch (2005) tested the carbon autonomy of fruit-bearing branches in three broadleaved species (i.e. *Carpinus betulus*, *Fagus sylvatica*, *Tilia platyphyllos*) by girdling branches at their base and maintaining the leaves. The seed mass remained unchanged with respect to the control, supporting the branch autonomy hypothesis. Moreover, it resulted that fruit production in masting tree species (i.e. *C. betulus*, *F. sylvatica* and *Quercus petraea*) is not strictly dependent on stored carbon reserves (Hoch et al., 2013). The idea that stored resources are not the key factor to support masting, suggests that the fruiting process might be more dependent on current year photoassimilates. The photosynthetic surface of a single branch (leaf area) might thus be the leading factor for accomplishing full production and maturation of fruits, even in years with a high seed production (Henery and Westoby, 2001).

The carbon autonomy of branches was partially supported by a study on *Ilex aquifolium* (Obeso, 1998). However, in the same work on *Ilex*, evidence emerged for carbon import in un-girdled but defoliated branches. This suggests that branches are only partially autonomous in terms of carbon and the connection with the main stem plays an important role during disturbances.

At the same time, one of the pioneers of carbon autonomy of branches highlighted the necessary interdependence of branches with the main stem and other branches due to competition for the same resources such as light and soil nutrients (Sprugel, 2002). Furthermore, it was recently suggested that the dynamics of carbohydrate translocation might follow significantly different patterns of priority in mature trees and seedlings (Hartmann et al., 2018). Thus, a debate is still going on regarding the translocation of carbon assimilates in the fructification stage of woody plants.

Because of its characteristics (large seeds, accessible crowns, masting behavior and commercial interest), *C. avellana* is the perfect candidate for testing the hypothesis of carbon autonomy of fruit-bearing branches. We hypothesized that carbon reserves in the stem of this species were an essential component for the maturation of fruits on terminal branches. The easiest technique to test the dependence of fruit maturation on current photoassimilates or stored carbon reserves is the application of a combined treatment of girdling and defoliation on fruit-bearing branches (Ichie et al., 2005; Obeso, 1998). The combination of defoliation with girdling was adapted from the model presented by Hoch (2005). We planned an experiment based on the manipulation of the carbon source-sink on fruit-bearing branches. If branches are fully autonomous, we will observe no depletion of fruit mass when the branch is isolated (i.e. girdled) from the main stem. On the contrary, if branches depend on carbon imported from the main stem, we will observe a reduction of fruit mass in the girdling treatment. Considering this last option, we could conclude that the masting behavior is somehow influenced by the presence of stored reserves and fruit-bearing branches are, thus, not carbon autonomous.

Materials and methods

The study was conducted during the summer of 2017 in Cancon, France in an orchard of *Corylus avellana* cv. Tonda di Giffoni planted in 1999. Nuts normally grow in the external part of the tree crown and the shells gradually lignify from June onwards. Nuts arrange on branches as single fruits or in clusters from two to four, each wrapped in green, wide bracts that desiccate at maturity (September-October). Thus, we selected the fruit-bearing branches on which we randomly applied one of the four treatments (Figure 3.4): fully defoliated and girdled (D_G), not defoliated and girdled (ND_G), fully defoliated and not girdled (D_NG) and control (Control). We had 18 replicates per treatment for a total of 72 branches sampled.

The branches were separated from the main stem (girdling) in late June 2017, phenology phases 78-79 (start of kernel filling) of the BBCH scale (Meier, 2001), by removing 5 mm wide strip of bark and phloem with a cutter at the base of the selected branches (mean diameter at the base 6.6 mm). The girdling was applied at the base of the fruit-bearing branch towards the stem, so that no leaves remained excluded. Exposed tissues were protected with Parafilm® in order to prevent desiccation. During the experiment, we checked that no regeneration of girdled tissues could occur. Leaves from the defoliated treatments were clipped, conserved in plastic bags, and labelled with the treatment code ID. Clipped leaves were then scanned in the lab and the leaf area was calculated with the software ImageJ.

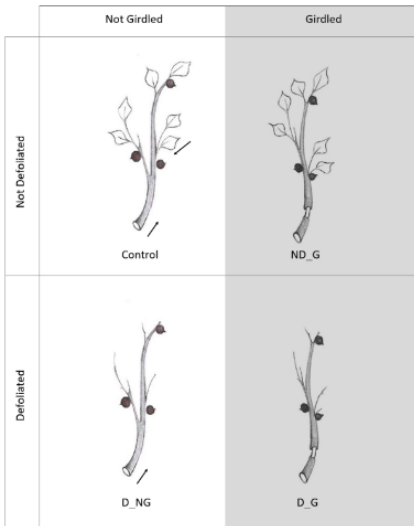


Figure 3.4. Schematic view of the four treatments applied to randomly selected branches. Arrows show the contribution of different carbon sources (leaves, branch tissues and imported resources from the main stem) to the fruit survival.

During the experiment, the size of nuts on selected branches was measured with a caliper and recorded. Before the nut fall, fruit-bearing branches were wrapped in organdy bags in order to keep the fruits of each treated branch separate and prevent fruit dispersal to the ground. Thus, at the end of the growing season, the survived fruits were

collected and divided by treatment. The wet and dry mass were calculated on both full fruits and seeds (cracked fruits without shell). The drying process lasted 15 hours in an oven at 40 °C.

The survival rate (Krebs, 1999) of fruits is calculated as: $FS_{final}/FS_{initial}$, respectively the fruit set at the harvesting time (final) and the fruit set at the beginning of the experiment (initial).

In parallel, we estimated the whole tree leaf area and collected the fruit mass per individual tree included in the sample plot (15 trees). The leaf area was derived through an ad hoc allometric equation built for *C. avellana* that correlates stem diameter with leaf area through dry matter analysis of both leaf and stem tissues (Pisetta, 2012). All branch diameters were measured in the field, while fruits were collected manually tree by tree on three consecutive harvests to follow their natural maturation. At each harvest, fruits were preserved in labelled bags with the identification code of each tree. They were then dried with the same procedure as that described for branches and weighed separately by mother tree.

Statistical analysis

To detect the differences between treatments on fruit growth, fruit survival, dry fruit mass, dry seed mass, we used one way ANOVA with Tukey post hoc multiple pairwise-comparison test. Relationships between leaf area and fruit mass were fitted to linear regression. All statistical analysis and visual representations were conducted using the software RStudio version 3.5.1 (2018).

Results

Verification of the dataset homogeneity

The most important morphological parameters measured on branches, i.e. leaf area (LA), branch length (BL), branch diameter (BD) and initial fruit set ($FS_{initial}$) did not differ across treatments (Table 3.1).

Table 3.1. Mean values ($\pm SE$) of leaf area (LA), branch length (BL), branch diameter (BD) and initial fruit set ($FS_{initial}$) across the treatments named as Control, defoliated and girdled (D_G), defoliated and not girdled

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(D_NG), non-defoliated and girdled (ND_G). All parameters were shown to be normally distributed (Shapiro test, $P > 0.05$), Letters show the grouping with a $P < 0.05$, Tukey post hoc tests.

Treatment	N. Branches	La (cm ²)	Bd (mm)	Bl (cm)	F _S ^{Initial} (n.)
Control	18	1581.04 (217.17)a	6.67 (0.48)a	60.00 (5.51)a	7.22 (1.11)a
D_G	18	1490.56 (147.25)a	6.14 (0.34)a	60.49 (4.05)a	5.00 (0.74)a
D_NG	18	1678.30 (167.79)a	6.97 (0.36)a	63.47 (3.96)a	7.22 (0.92)a
ND_G	18	1963.22 (195.50)a	6.67 (0.29)a	68.50 (4.02)a	6.78 (0.74)a

Verification of fruit growth across treatments

Fruit growth as diametric variation was measured from June to August just after the shell reached its final size. The final diameter was significantly different among all treatments ($P < 0.05$) except Control and ND_G, which had a similar final diameter (mean 20.6 mm and 21.1 mm respectively, $P > 0.05$). The treatment D_NG had the highest increase in fruit size over the period (8.5 mm), while the treatment D_G had a sudden drop in nut size after July 25th, resulting as being the treatment with the smallest fruits (Figure 3.5).

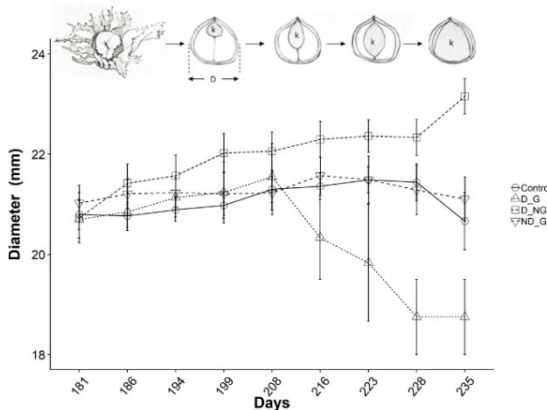


Figure 3.5. Mean values of nut diameter in mm from day of the year (DOY) 181 (June 28th) to 235 (August 21st) for all four treatments. Pictures on top show the development phases of the kernel (k) within the shell from shell formation (left) to the filling of the whole shell with the kernel at maturity. The process of kernel filling was strongly affected by the manipulation of carbon sink-source in the sampled branches

Manipulation of carbon sink-sources

Only the D_G treatment was significantly affected by the treatment (Fig. 3.6; $P < 0.05$). Mean survival in the Control was 0.67 (SE=0.08) and the median value was 0.71 with a large variation among samples. The survival rate was significantly different between treatments D_G and D_NG ($P < 0.05$), while the control was shown to have a survival not significantly different from treatment ND_G ($P > 0.05$).

All treatments accounted for a minimum 15% of dead fruits from the beginning of the experiment to the harvesting, including the Control. The most important effect of the manipulation was that girdling always decreased the average dry fruit weight of survived fruits compared to the Control (1.19 g in D_G and 1.62 g in ND_G) while the effect of defoliation alone is negligible (mean 2.20 g and 2.37 g) (Figure 3.7A).

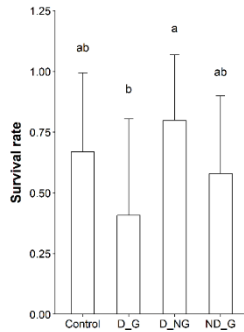


Figure 3.6. Survival rate across treatments, where the maximum value corresponds to high survival (1=100% fruits remained on the tree from the beginning of the experiment to harvesting). Values are means \pm SE. Letters above the bars show the grouping with a $P < 0.05$ performed with Tukey post hoc tests

Empty shells, i.e. missing seed at cracking, were counted in all treatments as a share of the final fruit set and ranged between 7.22% in the Control and 75% in D_G treatment (Table 3.2.). Because some fruits were found to be empty when cracked (21% over all treatments), it is important to consider the dry mass of seeds only (or kernel), excluding the shells (Figure 3.7 B). Similarly to total seed weight, the treatment negatively affected only the girdled branches while the defoliated branches did not differ from the Control.

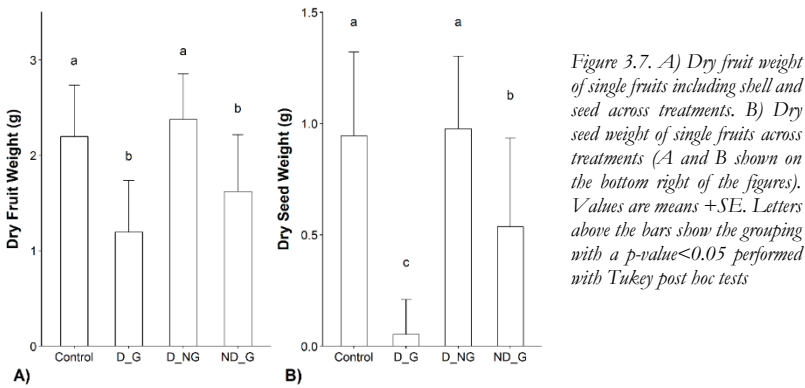


Table 3.2. Number of fruits at the beginning of the experiment $FS_{initial}$; % of dead fruits over the $FS_{initial}$; % survival rate; % of blanks, i.e. empty fruits over the FS_{final} ; % of full fruits over the FS_{final} ; dry fruit weight (DFW) including shell and kernel; dry seed weight (DSW) after cracking. DSW and DFW are expressed as mean value (\pm SE) in g.

Treatment	$FS_{initial}$ i (n.)	% dead	% survival	% blanks	% full	DFW (g)	DSW (g)
Control	130	30.77	69.23	7.22	92.78	2.20 (0.13)	0.94 (0.09)
D_G	90	66.67	33.33	75.00	25.00	1.19 (0.16)	0.05 (0.04)
D_NG	130	20.00	80.00	12.50	87.50	2.37 (0.12)	0.98 (0.08)
ND_G	122	48.36	51.64	17.43	82.57	1.62 (0.14)	0.53 (0.09)

Mean dry seed mass in the Control and D_NG resulted as being the highest (0.94g and 0.98g respectively), but ND_G had a significantly lower mean (0.54g). Treatment D_G had the lowest mean value of seed dry mass (0.05g). In this treatment, almost all fruits that were still on the branch at harvesting time were completely empty or with dead seeds (wrinkled or rotten). Only two fruits survived with an average seed dry mass of 0.47g.

Allometric relationships

The correlation of total leaf mass with total fruit dry mass (Figure 3.8) was revealed to be poor ($r^2 = 0.16$, $P > 0.01$) when single branches were considered.

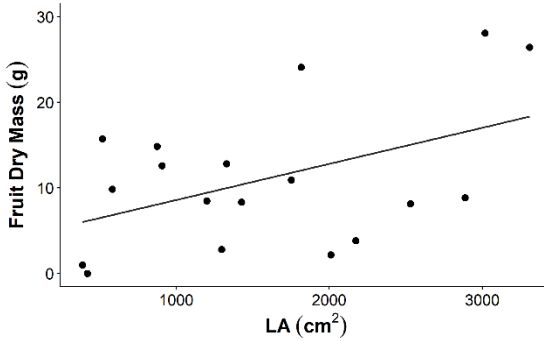


Figure 3.8 Branch leaf area (LA, cm²) against branch fruit dry mass in the treatment Control during the growing season 2017.

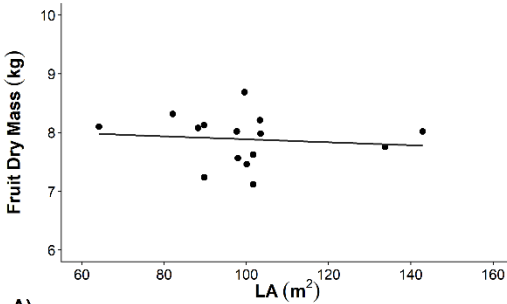
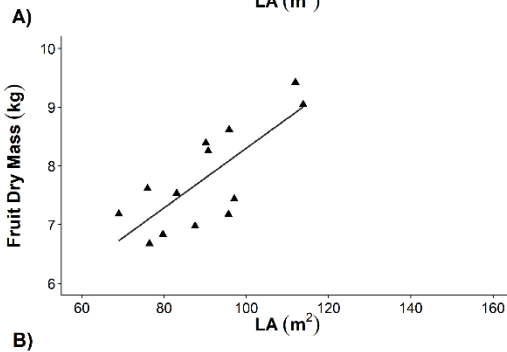


Figure 3.9 Leaf area (LA, m²) against fruit dry mass at the whole tree level in growing season 2016 (A) and (B) 2017 (A and B shown on the bottom right of the figures).



When the whole tree was considered, the results appeared different: during a masting year (e.g. 2016, Fig. 6.A) the correlation was again weak ($r^2=-0.06$, $P>0.01$), but in a non-masting year (e.g. 2017, Figure 3.9 B), the correlation increased significantly ($r^2=0.56$, $P<0.01$).

Discussion

With this study it emerged that in *C. avellana* cv. ‘Tonda di Giffoni’, the final fruit set is dependent on multiple carbon sources that include leaves, other branch tissues (i.e. radial parenchyma and photosynthetic fruit bracts), and imported resources from the main stem or other branches.

From the measurement of the diametric variation of fruits over the season, we observed that nut enlargement continues close to the harvesting. The highest increase was found in treatment D_NG, which suggests that fruits and their bracts acted as sinkholes for water and nutrients translocated from the rest of the tree. While Control and ND_G diameter growth was comparable but negligible from the beginning to end of the experiment, D_G had a sudden drop in growth after 20 days from the start of the experiment. This would suggest that, when leaves and stem resources are excluded, the remaining branchlet resources provide about 20 days of autonomy before fruit set is affected.

During the treatment period many fruits were abandoned by trees. In hazelnut, the occurrence of fruit mortality before maturity is a common phenomenon that increases when trees experience water stress during key phases of the maturation process (Cristofori, 2005). However, sampled trees were constantly irrigated from June to August in order to maintain the soil water content close to field capacity. We can thus exclude that the mortality observed during the experiment was caused by lack of water during the maturation process.

The survival rate at harvesting time was generally similar across all treatments, even if the Control and the un-defoliated and girdled branches resulted more similar to each other (D_NG, i.e. the check for carbon autonomy). In these treatments, both fruit sets benefit from the contribution of carbon from leaves and branch reserves, but only the Control is also supplied by the stem (or other branches) stores. However, this additional source was not enough to make the difference. Only D_G and D_NG resulted as having significantly different survival, showing that when defoliation occurs stem imported resources have a significant impact in ensuring fruit survival.

However, the non-significant correlation at branch level between LA and final fruit set confirms that branch LA alone might not be a predictive parameter of final fruit mass as was previously found by Obeso (1998) in samples of *Ilex aquifolium*. Furthermore, in *C. avellana*, the number of nuts also depends on other processes typical of anemophilous species. The differentiation of female flowers or the pollination success, which, in particular, appeared to be a very stochastic process due to pollen concentration in the air and wind transport, as well as weather conditions (Dąbrowska, 2008; Novara et al., 2016; Whitehead, 1969), are main driving factors for the number of fruits. Also some varieties such as cv. Tonda di Giffoni present a translocation heterozygote, which limits the number of successful nuts (Salasses and Bonnet, 1988). Moreover, the female flowers and receptivity of the stigmas, occur very early in the season in hazelnut, from the end of January to the end of February. In this period, it is not rare that the female flowers suffer from exposure to abnormal temperatures before the nut filling starts (Solar and Stampar, 2011). Besides, depending on the variety there is a percentage of cluster drop before fertilization. This phenomenon is closely related to apical dominance, which occurs along the one-year-old shoot and the peduncles of catkins (Germain, 1994). Moreover, cluster drops that occurred in June have been also reported to be associated with

nutritional deficiencies and lack of fertilization. In these cases ovules could not develop in non-fertilized ovaries (Milošević and Milošević, 2012). All these factors can negatively affect final fruit set and explain why the survival based on fruit number ratio did not clearly differ between treatments.

The cracking of survived fruits highlighted the occurrence of empty ones in all treatments. Empty shells (blanks) are also a frequent trait in hazelnut crops. In a multi-cultivar study on *C. avellana*, Mehlenbacher et al. (1993) found that blanks account for about 13% of the total yield in cv. ‘Tonda di Giffoni’ under standard growing conditions. Our findings on the Control showed an occurrence of only 7.22% of final yield, suggesting that growing conditions are good in the site. As expected, the amount of blanks increased in the other treatments due to the lack of carbon sources, reaching a peak in D_G with 75%. Interestingly, despite treatment D_NG having the highest survival, it also showed more blanks compared to the Control (Table 2). On the other hand, the filling process of shells during maturation is partially linked to the carbon imported from the main stem as can be seen in the un-defoliated and girdled treatment where blanks increased. In the defoliated and girdled treatment, almost all of the survived nuts were blanks, while only 10% of the initial fruit set were full.

C. avellana tends to bring only well-formed fruits to maturity, while the rest are abandoned along the maturation process. Indeed, the average seed dry weight of survived full fruits is similar to the Control in the defoliated and un-girdled branches. This confirms that the defoliation did not lead to decreased seed mass (Obeso, 1998; Hoch, 2005) as long as the capacity of fruit-bearing branches to import carbon resources is maintained. Rather we even observed some overcompensation of carbon import due to leaf loss. This outcome suggests that the stem tissues and leaves on other non-fruit-bearing branches are a necessary contribution to ensure maximum nut productivity.

Accordingly, if we upscale the phenomenon from the branch to the whole tree, the correlation found between LA and fruit mass becomes significant and suggests that the tree fruit mass is either led by the total tree LA or by the stem size (stem resources). The stochastic phenomenon that affected the weak correlation on branches appears to be buffered when the entire tree is considered. Overall, this would suggest that the plant is relatively resistant to disturbances and that it is able to use different sources of C (i.e. from other stems or from leaves) in order to maintain the potential seed production. However, this correlation between whole tree seed mass and leaf area was conserved only during the non-masting year of 2017. Indeed, in 2016 (masting year) the total tree seed mass was completely unrelated to the tree leaf area. Thus, even if trees have a general balance between photosynthetic and reproductive mass, in masting years the additional yield that cannot be coupled to an increase of leaf mass will be supported by stored reserves. This observation would be in agreement with the depression of vegetative growth observed in “low-yield” years in many deciduous fruit trees (Guerrero et al., 2006).

The exclusion of stem resources by girdling significantly decreased the dry seed mass in defoliated (D_G) and un-defoliated (ND_G) branches. The significant decrease of dry seed mass in ND_G compared to the Control suggests that branch resources were not sufficient to support the full maturation of kernels. From this perspective, only observing the fruit weight (shell+kernel) might be misleading for assessing maturation success. In contrast to other orchard fruit trees such as nectarines (Rivas et al., 2007) or avocado (Lahav et al., 1971), hazelnut does not benefit from an ameliorative effect on fruit yield derived by the application of girdling on fruit-bearing branches. Interestingly, in the treatment D_G the few full fruits conserved a dry mass similar to that in the treatment ND_G. This suggests that there must be a source of carbon supply for these fruits that is not delivered either by the branch leaves and or by stem

reserves. A possible explanation for their successful filling might be the presence of photosynthetic bracts wrapping the seed, which could have provided enough carbohydrates to act as a few leaves. Indeed, the contribution of photosynthetic bracts to seed maturity has already been reported in *Carpinus* (Hoch and Keel, 2006; Hori and Tsuge, 1993). However, full shells in D_G could be also explained by the presence of reserves stored in the same branch, for example as axial parenchyma. In fact, in the genus *Corylus*, concentrations of aggregate rays in branches were observed as a typical trait (Crivellaro and Schweingruber, 2013). Despite that, these reserves were shown to be insufficient to maintain the large majority of the initial fruit set to the end of the season.

Conclusions

With this study we have shown that, during the fruit maturation process in hazelnut, fruit-bearing branches are not fully carbon autonomous when long lasting treatments are applied. Indeed, we found higher average seed dry mass in the control respect to the un-defoliated and girdled branches, which suggests that a fraction of carbon for fruit production is imported from the main stem or neighbor branches. Despite that, we noticed that about 20 days are needed for the fruits to shrink and fall after the application of girdling and defoliation. This leads to question if branch resources can actually support fruits for a limited time (two weeks in hazelnut) and weather this depends on the size and turgor of tissues. More research in this direction may be useful to give indications on irrigation management close to the maturation stage. The idea of branch autonomy on fruiting shoots was also weakened by the missing correlation between branch leaf area and total branch fruit mass. This is confirmed by the stronger correlation between tree leaf area and fruit mass in non-masting compared to masting years that opens once again the possibility that the increased yield in masting years probably relies on stored resources

which exceed the current year photoassimilates. These observations might lead to reconsider management strategies in hazelnut orchards to encourage resources storage in wooden tissues of the stem and main branches, while limiting the pruning in non-masting years.

FINAL CONCLUSIONS

This study aimed at providing physiologically based information about the transpiration of mature trees in hazelnut orchards. The final addressees of these outcomes are a variety of orchard managers, who are located in different climatic settings. Thus, the rationale that accompanied us in developing this study was mainly practical.

First of all, we tested the relative response of transpiration to one of its main drivers: the vapor pressure deficit of the atmosphere. The transpiration increased linearly until 20 hPa (classes of instant values) in all areas, while it became less sensitive at higher VPD values. However, considering mean daily VPD, the threshold value decreases often earlier, around 10 hPa (Pasqualotto et al., 2018). The second main driver for transpiration, the radiation intensity, positively affected the response of transpiration to the vapor pressure deficit. Orchards located in the Southern hemisphere had a relatively quicker transpirative response to VPD. No difference in the transpiration *vs.* VPD was measured between cultivars Tonda Gentile delle Langhe and Tonda di Giffoni in Chile, despite the findings of Cincera et al. (2018) reported differences measured on young seedlings of the same cultivars. The absence of cultivar effect on transpiration patterns might have several causes. First, the genotypic selection on hazelnut is loose, so that the same cultivar used in the two experimental design might not have exact genotypic features. Another reason might be the acclimation to the Chilean environment. At last, and maybe the most important reason, is the sampling on mature trees in the field. To these individuals belong only few outer leaves subject to direct radiation, while many others are under lower light intensity within the crown. Indeed the bending point of the response curve occurs at much lower VPD values in our field experiment respect to seedling leaves. Additional irrigation (double drip line and drip line plus sprinklers) seemed to have no effect in changing the transpiration response pattern, while half irrigation had controversial effects respect to the standard. In the latter case, we

suspect malfunctioning of the irrigation system, which was based on punctual soil water content information.

The calibration of Granier's thermal dissipation probes combined with the potometer and the liquid flow meter had a double intent. First, to derive an equation to correct the underestimation of sap flow, which is commonly reported in this type of sensors. This step served to link the transpiration loss at different VPD to the leaf area of the tree. Indeed, this allows knowing the range of water lost per unit of leaf area based on mean daily VPD conditions with the final aim to reintegrate this amount with irrigation. This approach is meant to be flexible according to different orchard features. Because the leaf area increases with age and orchard density, the leaf area based transpiration can be up scaled to the average leaf area at the tree or hectare unit with a simple multiplicative approach to fit the commands of the irrigation schedule.

Still, the calibration is performed assuming that the whole sap wood area has relatively constant functionality in a conservative perspective for managing the irrigation. This assumption was made by the observation of the colored cross sectional area produced by the fuchsine perfusion, which suggested that water was transported across the whole area homogeneously and no specific regions were clearly inactive, except for the parenchyma rays. The 25% of the sap wood area of parenchyma was subtracted from the count of the active sap wood area as recommended for non-active tissues (Paudel et al., 2013). Various authors reported radial sap flow decrease with sapwood depth after a peak below the cambium on both conifers (Cermák et al., 1992; Delzon et al., 2004; Ford et al., 2004; Oren et al., 1999) and broadleaves (Berdanier et al., 2016; Gebauer et al., 2008; Nadezhdina et al., 2002; Poyatos et al., 2007). Ring-porous species would have a much sharper decrease respect to diffuse porous (Berdanier et al. 2016). However, radial decrease with wood depth was reported to be highly variable. Variation of the soil water content (Fernández et al., 2001), stomata closure on the outer layer of the crown (Fernandez et al. 2001; Ford

et al., 2004; Hernandez-Santana et al., 2016; Poyatos et al., 2007a), as well as sample diversity (Steppe et al., 2010) and day-time (Ford et al., 2004), would cause the shift of the decreasing pattern towards inner xylem to prevent cavitation. Because of these numerous uncertainties and of the focus on assessing irrigation requirements, no correction about the sap flow profile was applied here, yet. In this way, we expect to avoid the risk of underestimating again the actual sap flow and of under-irrigating the orchard.

A further objective of this study was to consider the relation between VPD and canopy conductance to seize the limiting condition for the potential reproductive biomass. Unfortunately, it is difficult to get reliable data of productivity on extensive surfaces, either because of yield fluctuations between consecutive years or because of technical issues (multiple harvesting, uncertain weighting techniques). Thus, we used the canopy conductance variation with VPD as a proxy for understanding the conditions of potential carbon uptake of trees. There is an interval of VPD (on average between 5 and 20 hPa) in which the relative canopy conductance is higher during the growing season. The higher the frequency of VPD values typical of this interval, the higher the chances for carbon uptake. Also, we observed that during the central months of the growing season, trees are in a non-optimal condition respect to the potential conductance of the species (on average about 50%). These indications may contribute to build suitability maps for the species and help decision makers to select appropriate growing areas. The ideal will be to increase the frequency of high canopy conductance or the integral of total conductance occurrences at the site level. The manipulation of the canopy structure to lower the VPD gradient of free atmosphere air *vs.* leaf, as well as irrigation, might be concrete field actions to test improvements.

Finally, we aimed at understanding the link between reproductive and vegetative biomass. With the experiment on manipulation of carbon sources and sinks, we tested to what extent leaves (and thus current transpiration) are determining the survival and seed dry mass compared

to stem resources. It resulted that at the branch level the leaf area represents an important, but not exclusive, support for yield success. At the same time, the leaf biomass at the tree level seemed well correlated to the total tree fruit mass, but only in non-masting years. These results suggest that carbon uptake estimated from leaf based data are generally valid but might underestimate the actual need of carbon resources to support masting events. Still, is to be considered that the reproductive effort in hazelnut may be actually distributed among agamic and gamic strategies (Reekie and Bazzaz, 2000). Thus, more studies shall investigate the allocation to these structures, if they alternate the carbon sink and to which proportion respect to the main vegetative biomass (main trunks and leaves).

ACKNOWLEDGEMENTS

This research has been carried out thanks to the cooperation and economic contribution of the Hazelnut Company, Division of Ferrero Group, which promotes applied research in the agronomic field.

In addition, I would like to thank all the people who supported me in accomplishing the PhD process.

Prof. Tommaso Anfodillo, who was always keen to provide advice and to discuss ideas and results. Tommaso de Gregorio and Eloy Suarez Huerta from the Ferrero Hazelnut Company, who trusted me for this project and shared their knowledge, human and logistic support in all the places we visited for this project. A special thanks to Dr. Vinicio Carraro, who provided essential technical support and field experience on sensors and data analysis. All the teams from experimental farms I visited: Fruticola Agrichile, AgriGeorgia, AgriAustralis, Unicoque-France and ANPN, and all private owners of the orchards that have been involved for providing their collaboration and support at the local scale. Thanks also to all the intern students who helped collecting field data.

Thanks to my colleagues, friends and family for listening and talking to me with patience and an open mind. A great thank to Daisaku Ikeda for ceaselessly encouraging and spurring me on to bring out value from all challenges. A final thanks to Thomas, who keeps me down to earth along this journey.

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