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## Apple tree growth and ramification in an agroforestry system

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Master's dissertation  
Agricultural Engineering Course  
Speciality: Sustainable crop production (PVD)

Apple tree growth and ramification in an agroforestry system



Benjamin PITCHERS

Hosting Research unit: UMR SYSTEM

Presentation year: 2017

Financial support: INRA, GIS FRUIT



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Benjamin PITCHERS

Presentation year: 2017

Master's dissertation prepared under the supervision of:

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Submitted the: 15/09/2017

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## Abstract

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Contemporary agriculture has to face new scientific and societal challenges (IAASTD 2009). Recent studies have shown that humanity will have to double agricultural production to meet the demand in 2050 (Ray et al. 2013). But agriculture needs to increase its production while addressing environmental concerns. Different solutions have been proposed in order to answer these challenges including new practices more resilient to external pressure (climatic disturbances, new diseases, economic crises) and more efficient (Foley et al. 2011).

Agroforestry is the association, on a plot, of perennial and annual crops and possibly animals (Dupraz & Liagre 2011). Agroforestry systems (AFS) in temperate climate are mainly composed of two layers: the tree layer and the herb layer. However, an original timber-tree-based AFS could be enriched with fruit-trees in an intermediate vertical stratum. In such context, the interest but also the challenges of timber- and fruit-tree based AFS lie in: (i) fruit production, (ii) microclimate and plant ecophysiology and (iii) biocontrol of pests and diseases (Lauri et al. 2016). The apple-agroforestry experiment (Growing AgroForestry systems with Apple, GAFA) was set in 2016, combining hybrid walnut trees planted in 1995, apple trees planted in March 2016 and 5-year-old Lucerne.

This work focuses on the effects of the distance between apple trees and walnuts, inducing presumably a gradient of competition, on various architectural and morphological aboveground features of the apple trees. The AFS reduced incoming light and global radiation but acted as a buffer on temperatures. These modification on the aboveground environment of the AFS induced a buffer effect on water potential daily evolution ( $\Psi$ ) as well as different growth strategy of the apple tree in their second year. There was a difference in the AF apple trees stem geometry (higher slenderness and lesser tapering), architecture (less ramifications), and morphology (higher leaf area and specific leaf area).

**KEYWORDS:** Agroforestry, microclimate, apple trees, architecture.

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## Résumé

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L'agriculture contemporaine doit faire face à de nouveaux défis scientifiques et sociétaux (IAASTD 2009). De récentes études ont montré qu'il sera nécessaire de doubler la production agricole mondiale pour répondre à la demande en 2050 (Ray et al. 2013). De plus, l'agriculture doit limiter ses externalités négatives. Le développement de nouveaux agrosystèmes plus résilients face aux pressions externes (réchauffement climatique, maladies, crise économique) et plus efficaces dans leurs utilisations d'intrants fait partie des solutions avancées (Foley et al. 2011).

L'agroforesterie consiste en l'association, sur une même parcelle, d'essences végétales pérennes et annuelles et éventuellement d'animaux (Dupraz & Liagre 2011). Les systèmes agroforestiers (AFS) en climat tempéré sont majoritairement composés de deux strates : la strate arborée et la strate herbacée. Cependant, un système agroforestier à base de bois d'œuvre peut être enrichi d'une strate verticale intermédiaire composée d'arbre à fruits. Les intérêts et les défis d'un tel système réside dans : (i) la production de fruit, (ii) le microclimat et l'écophysologie des plantes et (iii) le biocontrôle des ravageurs et maladies (Lauri et al. 2016). L'expérimentation a été mise en place en 2016 combinant des noyers hybrides plantés en 1995, des pommiers plantés en mars 2016 et une luzernière de 5 ans.

Ce rapport s'intéresse aux effets de la compétition aérienne avec les noyers sur l'architecture et la morphologie des pommiers. L'AFS réduit la lumière incidente et le rayonnement global en plus de tamponner les variations quotidiennes de températures. Ces modifications du microclimat induisent une moindre variation quotidienne du potentiel hydrique ( $\Psi$ ) ainsi que différentes stratégie de croissance des pommiers. Je montre ici des effets du contexte agroforestier sur la géométrie des pousses du pommier (élancement plus important et conicité moins élevée en agroforesterie), sur son architecture (moins de ramification axillaire en agroforesterie) et sur sa morphologie foliaire (surface et surface foliaire spécifique plus grandes en agroforesterie).

**MOTS CLES :** Agroforesterie, microclimat, pommier, architecture.

## Résumé étendu

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### *Introduction*

L'agriculture contemporaine doit faire face à des nouveaux défis scientifiques et sociétaux (IAASTD 2009). De récentes études ont montré qu'il sera nécessaire de doubler la production agricole mondiale pour répondre à la demande en 2050 (Ray et al. 2013). De plus, l'agriculture doit limiter ses externalités négatives (pollution, dégradation des sols...). Le développement de nouveaux agrosystèmes plus résilients face aux pressions externes (réchauffement climatique, maladies, crise économique) et plus efficaces dans leurs utilisations d'intrants fait partie des solutions avancées (Foley et al. 2011).

L'agroforesterie consiste en l'association, sur une même parcelle, d'essences végétales pérennes et annuelles et éventuellement d'animaux (Dupraz & Liagre 2011). Les systèmes agroforestiers (AFS) en climat tempéré sont majoritairement composés de deux strates : la strate arborée et la strate herbacée. Cependant, un système agroforestier à base de bois d'œuvre peut être enrichi d'une strate verticale intermédiaire composée d'arbre à fruits. Les intérêts et les défis d'un tel système résident dans : (i) la production de fruit, (ii) le microclimat et l'écophysiologie des plantes et (iii) le biocontrôle des ravageurs et maladies (Lauri et al. 2016). L'expérimentation a été mise en place en 2016 combinant des noyers hybrides plantés en 1995, des pommiers plantés en mars 2016 et une luzernière de 5 ans.

Mon objectif était d'étudier les effets de la distance entre les noyers et les pommiers, qui induisent un gradient de compétition, sur l'architecture et la morphologie aérienne du pommier. Plus précisément j'ai analysé :

- l'architecture (distribution topologique des types de bourgeons latéraux le long de l'axe porteur) et la géométrie de la pousse du tronc développée l'année précédente, i.e. première année de croissance,
- la dynamique de croissance (longueur et nombre de feuilles) du tronc pendant sa deuxième année de croissance,
- la morphologie des feuilles (surface foliaire et surface foliaire spécifique).

### *État de l'art*

Les systèmes agroforestiers, comme les systèmes plurispécifiques de façon générale, sont des agrosystèmes conçus pour optimiser, dans le temps et l'espace, l'utilisation des ressources (lumière, eau, minéraux) en maximisant les interactions positives et minimisant les interactions négatives entre les composants du système (Jose et al. 2004). Ainsi, en se basant sur différents indicateurs tel que le LER (Land Equivalent Ratio), il est possible de montrer que les agrosystèmes plurispécifiques peuvent avoir des rendements supérieurs aux agrosystèmes monospécifiques. Cependant la description et la compréhension des interactions prenant place au sein de l'agrosystème sont un préalable indispensable à sa conception et son optimisation. Ces interactions entre espèces peuvent être appréhendées dans un système agroforestier à partir des différents types de relations définies par l'écologie (Jose et al. 2004).

Il s'agit donc de concevoir et piloter le système agroforestier de manière à maximiser les synergies entre les différentes espèces et de minimiser la compétition pour les ressources et de réguler les bioagresseurs. L'objectif recherché est que la compétition interspécifique soit moins importante que la compétition intraspécifique. Dans un système agroforestier, ces interactions vont évoluer au cours de la vie du système. Les plantes pérennes vont croître au cours du temps et devenir de plus en plus

compétitives pour les plantes annuelles. Il est alors possible d'analyser les interactions en s'intéressant à deux compartiments distincts : les interactions aériennes et les interactions souterraines.

Au niveau aérien la seule ressource pour laquelle il y aura de la compétition est la lumière. De plus la compétition pour la lumière évoluera avec l'âge du système agroforestier. L'ombre portée des strates les plus hautes (relation d'amensalisme) réduira le rayonnement reçu par les strates inférieures mais aura également un effet sur la régulation du vent et des températures extrêmes sur ces mêmes strates. Enfin, la présence d'un linéaire arborée peut servir de refuge pour les auxiliaires mais également les ravageurs généralistes (Jose et al. 2004; Jose et al. 2006; Ong et al. 1991; Forey 2016).

Dans un environnement ombragé où l'interception de la lumière est essentielle les plantes auront de plus longs axes et entrenœuds (Kami et al. 2010), des feuilles avec une surface foliaire plus grande (Lambers & Poorter 1992), une surface foliaire plus grande et une réduction du rapport croissance souterraine/croissance aérienne (Grime 1977).

Les interactions souterraines entre les arbres et la culture en système agroforestier sont directement liées à l'occupation spatiale des racines. Dans un cas de complémentarité optimale, où les racines des deux cultures prospectent des volumes de sols différents, nous aurons moins de compétitions que dans le cas où les racines occupent des volumes de sol identique (Gliessman 1985). Ces interactions sont nombreuses et difficile à appréhender que ce soit la compétition pour le prélèvement de l'eau et des nutriments ou des facilitations (ascenseur hydraulique, exsudats racinaires).

NB : les possibles effets de la compétition souterraine n'ont pas pu être traités de manière satisfaisante en 6 mois.

### *Problématique et hypothèses scientifiques*

La problématique à laquelle nous essayons de répondre n'est pas tant de savoir si il y a des différences dans la croissance aérienne entre des pommiers en système agroforestier et des pommiers en verger traditionnel que de voir où ces différences se situent.

Ayant considéré que l'intensité des interactions dans le système agroforestier peut être corrélé à la distance entre les plantes et surtout les noyers qui, nous supposons, seront à l'origine de la majorité des interactions aérienne nous avons formulé deux hypothèses,

- l'environnement aérien diffère dans un système agroforestier mature (noyers âgés) comparé à celui d'un verger traditionnel,
- les interactions aériennes (microclimat et ombre portée par le noyer dans la strate supérieure) peuvent influencer la croissance aérienne du pommier et sa ramification,

Afin de s'assurer que les différences que nous pourrions observer étaient dues aux interactions aérienne dans le système agroforestier nous avons fait en sorte de limiter au maximum les autres facteurs limitants. Pour cela les pommiers ont été irrigués et fertilisés et nous sommes intervenus sur les maladies et ravageurs qui auraient pu affecter la croissance aérienne du pommier.

Nous avons réparties nos 148 pommiers dans deux blocs afin de capter l'hétérogénéité de la parcelle et trois modalités composés du témoin agricole (AC), l'inter-rang du système agroforestier (AF\_IR) et le rang du système agroforestier (AF\_R).

### *Matériels et méthodes*

La parcelle agroforestière choisie pour l'expérimentation est la parcelle A1 du domaine de Restinclières situé dans le sud-est de la France sur la commune de Prades-le-Lez (34730).



Nous avons caractérisé l'environnement aérien et le microclimat dans chaque modalité à l'aide de photo hémisphérique (gap fraction), de capteurs d'humidité, de températures et d'hygrométrie (sonde HMP 155) ainsi que des pyranomètres (SP 110). Nous avons également mesuré l'effet de la modalité de croissance du pommier sur le potentiel hydrique des plantes à l'aube ( $\Psi_{pd}$ ) et au midi solaire ( $\Psi_{md}$ ) à l'aide de deux chambres à pression.

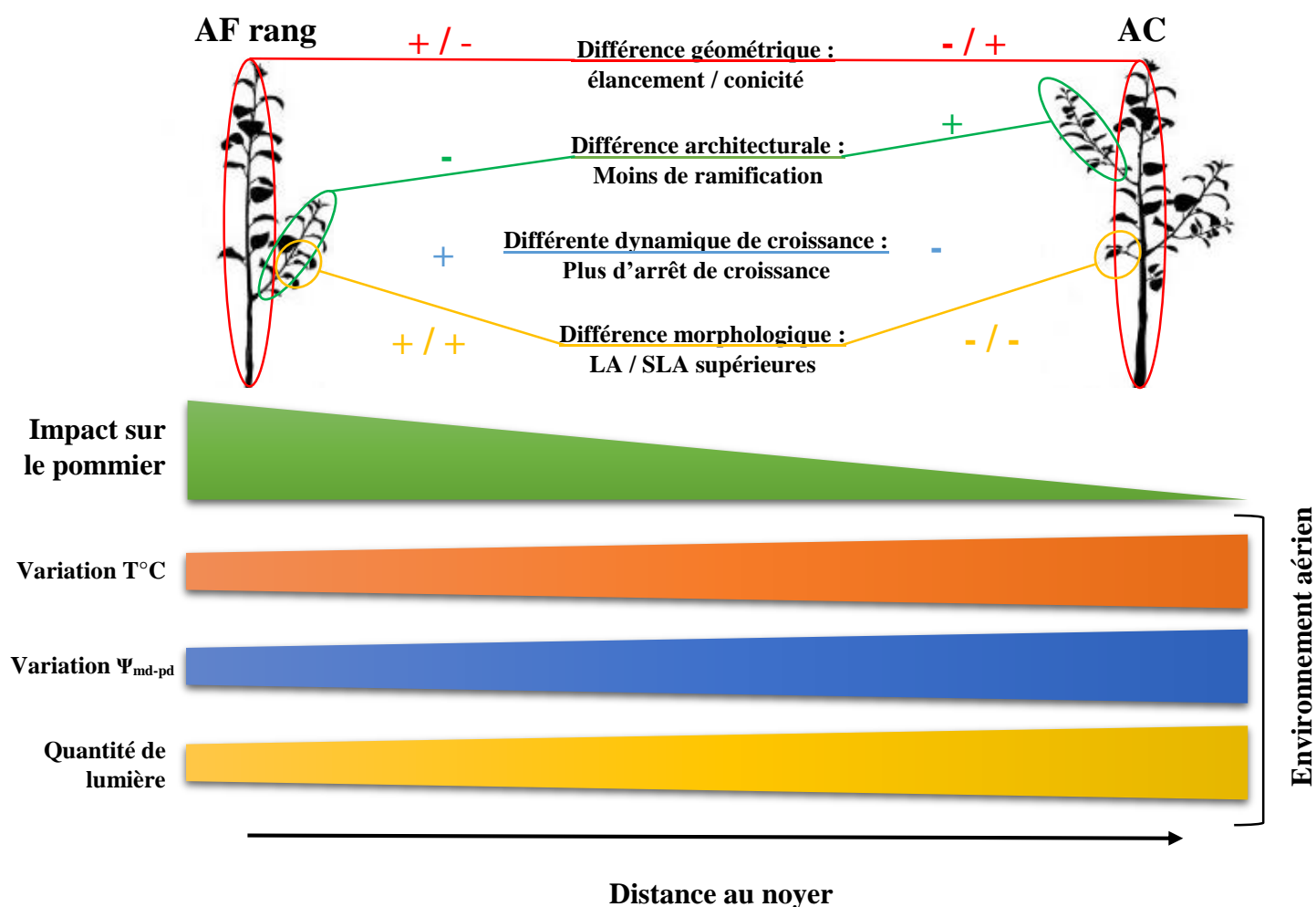
Les mesures de longueur et de diamètre du tronc âgé d'un an (1<sup>ère</sup> année de croissance, 2016) ont été faites avec un pied à coulisse et un mètre à mesurer.

Sur ces mêmes troncs, l'analyse de la ramification axillaire a été réalisée en notant nœud à nœud la nature des axillaires (inflorescence, végétatif, latent, extinction) développés en 2017. Pour les inflorescences, le suivi phénologique a également été réalisé du stade débourrement au stade nouaison.

Les surfaces foliaires ont été mesurées à l'aide du logiciel Winfolia® sur quinze feuilles par modalités qui ont ensuite été mise à l'étuve pendant 48 heures à 60°C avant d'être pesées pour calculer la surface foliaire spécifique.

### Résultats

Les résultats sont synthétisés dans le schéma suivant :



### *Discussion*

Les résultats obtenus sont conformes à ce qui avait déjà été rapporté dans la littérature sur la croissance de plantes pérennes dans un environnement lumineux restreint. De plus, nous n'avons pas observé de différence dans le plastochrone entre les pommiers des différentes modalités. Quand les pommiers poussaient, ils poussaient de manières identiques. Nous faisons l'hypothèse que les différences de croissance relative et longueur du tronc étaient dues à la longueur des entrenœuds les premières semaines et dans la proportion d'arrêt de croissance environ un mois après le débourrement des noyers.

Un des résultats observés peu discuté dans la littérature concerne l'effet tampon du système agroforestier sur la variation quotidienne du potentiel hydrique. Nous voyons trois explications possibles à cet effet : (i) le système agroforestier limite le VPD (le pommier étant considéré comme isohydrique) (Lakso 2014), (ii) les pommiers en système agroforestier limite leur transpiration dès l'aube et (iii) il y a plus de mouvements hydrauliques dans le sol « sous les noyers » (ascenseur hydraulique) limitant la baisse du potentiel hydrique du sol pendant la journée.

Il y a donc différents effets du système agroforestier qui influent sur la stratégie de croissance des pommiers. Certains de ces effets ne sont apparentés qu'à un effet de l'ombrage du noyer. Cependant, il y a probablement également un effet des interactions racinaires sur la croissance du pommier. L'hypothèse formulée autour de l'idée d'ascenseur hydraulique qui influencerait sur le potentiel hydrique du sol au midi solaire va dans ce sens.

D'un point de vue méthodologique il sera important pour les futures analyses de repenser le système de caractérisation du contexte de croissance des pommiers, actuellement limité à la « modalité », via l'utilisation d'un indicateur plus intégratif (Fichtner et al. 2017). En effet, la répartition des pommiers en différentes modalités nous semble trop simpliste. De plus, il n'a pas été possible de s'assurer qu'il n'y avait pas d'effet des interactions souterraines en particulier de compétition pour l'azote. La compétition souterraine a souvent des effets plus importants sur le développement des plantes que la compétition aérienne (Wilson & Tilman 1991).

N'oublions pas que l'objectif des arbres fruitiers reste la production, il sera donc intéressant de voir si il y a des différences au niveau de la production dans les années à venir et comment les différences de croissances vont impacter le rendement quantitativement et qualitativement.

## Acknowledgements

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This project has been funded by the GIS Fruit, I greatly appreciate the support, and trust that the knowledge we have gained from this work will more than adequately reward them for their investment.

My supervisor, Pierre-Éric Lauri, has been of great assistance during this six month work experience and his addition to this whole work is certainly far greater than I mention here. Thank you for letting me work on this project and your guidance.

The agroforestry system on which we worked in this project was situated in the domaine de Restinclière. As so I thank the department of Hérault for letting us use these lands and all the members of the team involved in the management of the plots. Thank you Lydie Dufour, Alain Sellier and Jean-françois Bourdoncle for your support and advice.

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This experience would not have been the same without all the others interns that shared this time with me. A special thank-you to Anaïs for all these shared long days in the field doing measurements.

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## Foreword

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This work experiment was realised in the French national institute for agronomic research (INRA) at the end of my engineer's course in Montpellier SupAgro. Three different partners are involved in this work, INRA and GIS Fruit for scientific supervision and financial support, respectively, and Montpellier SupAgro for tutoring.

I worked with the “Tropical and Mediterranean cropping system functioning and management” joint research unit (UMR System) located at Montpellier SupAgro campus. It aims to produce knowledge and tools to assess and design cropping systems combining economic efficiency and the production of environmental services. UMR system is composed of four scientific teams:

- Associated Biodiversity and Ecosystem Services topic – BASE
- Transitions, Trajectories, Resilience topic – TTR
- Availability, sharing and use of resources (light, water and nitrogen) in multi-species systems – Resources
- Cropping Systems, Modelling and Testing team - Syme

The GIS Fruit is an association of scientific interest dedicated to the French fruit sector created in 2012. It is an original form of organization which draws together 22 French partners involved in research, extension and training, alongside with professional organizations in the fruit sector. GIS Fruits aims at developing a long term, joint strategy covering a range of activities in the sector, from research up to the transfer of innovations to economic actors.

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## Acronym

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GAFA: Growing AgroForestry systems with Apple

GIEE: Groupements d'intérêt économique et environnemental

GIS: Groupement d'intérêt scientifique

IFT: Indice de Fréquence de traitement

UMR: Unité mixte de recherche



## Introduction

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### *Agriculture at a crossroads*

Contemporary agriculture has to face new scientific and societal challenges (IAASTD 2009). Recent studies have shown that humanity will have to double agricultural production to meet the demand in 2050 (Ray et al. 2013). The demand increase will be caused by (i) demographic growth, the latest estimations situate us at roughly 8,9 billion human beings in 2050 (Cohen 2003), (ii) changes in diets (increase of meat consumption) and (iii) increase in bioenergy use (Cirera et al. 2010; Kearney 2010).

But agriculture needs to increase its production while addressing environmental concerns. Agriculture is behind many environmental problems such as climate change, biodiversity loss and degradation of land and freshwater (Foley et al. 2005). In fact, agriculture is one of the sectors driving the environment beyond the « planetary boundaries » (Rockström et al. 2009). Different solutions have been proposed in order to answer these challenges including new practices more resilient to external pressure (climatic disturbances, new diseases, economic crises) and more efficient (Foley et al. 2011).

To meet these stakes France adopted the « Loi d’Avenir pour l’Agriculture, l’Alimentation et la Forêt » in 2014 driven by the former minister of agriculture Stéphane LEFOLL. This law aims to develop agroecological practices by creating work groups (GIEE) and introducing agroecology lessons in agricultural schools. Looking at the practices, agroecology relies on ecological services to maximise production and manage of agrosystems. What agroecology is and should be is an endless controversy but everyone seems to agree that it should limit the use of inputs by diversifying agrosystems and crops (Altieri 1999; Vandermeer et al. 1998; Lin 2011).

Agroforestry is the association, on a plot, of perennial and annual crops and possibly animals (Dupraz & Liagre 2011). This agrosystem is considered agroecological since it aims to increase the efficiency of natural resources usage by diversifying the system and benefiting from the interactions between the different species (Gliessman 1985). Agroforestry systems in temperate climate are mainly composed of two layers: the tree layer and the herb layer. Even if these agrosystems are being studied, science has still many questions to answer before concluding on their agricultural interests.

Nowadays innovative agroforestry systems are being created on farms or in technical institutes, however, very few of them combine three layers: a high shrub layer, an orchard and an herbal layer. In this work we focus on the apple.

### *Integrating fruit-trees in temperate and Mediterranean agroforestry systems*

Orchards, and especially apple orchards, are responsible for leaching of chemical products/pesticides in the environment and are subject to thermal stress during summer in the south of France. Moreover, apple is the first fruit France produces with 1 600 000 tonnes in 2015 (Agrete 2017) but also the most sprayed with a treatment frequency index (IFT) of 35.1 in 2011 at a national scale (Agrete 2017). But diversified agrosystems are known to have some advantages of which: (i) to limit pest, (ii) to limit disease and (iii) to reduce the

consequences of climatic disturbances (Lin 2011). An agroforestry system which combines woody perennials, fruit-trees and herbaceous plants (annual crop and/or nitrogen fixing plants) could reduce the negative externalities of the orchard as well as offering different sources of income for the farmers. However, such an agrosystem needs to be studied beforehand starting with the growth and the development of apple trees in contexts of above- and belowground competitions. In fact, when the environment is modified so is the growth dynamics of the plants (Cleland et al. 2007). Apple trees fruiting capacity is multifactorial but is also dependent on resource supply which is related to vegetative growth (Corelli Grappadelli et al. 1994; Lauri & Corelli Grappadelli 2014). It is then important to follow the establishment of the apple trees the first years.

Our experiment was set in the Restinclières domain, belonging to the Hérault department, located at Prades-le-Lez in the south east of France. This domain, have 53 ha of agroforestry plantations making it an important site of study (<http://umr-system.cirad.fr/les-agrosystemes-etudies/systemes-agroforestiers-temperes-et-mediterraneens>). To fathom the interactions between perennial and annual plants and the agronomic interest of this system, many studies and theses have been conducted.

The apple-agroforestry experiment was set in 2016, combining hybrid walnut trees planted in 1995, apple trees planted in March 2016 and 5-year-old lucerne, with the objective to study the impact of this association on the apple tree establishment in a first phase, and production in a second phase (Appendix 0).

We consider that, at any given stage, the apple tree architecture reflects internal competitions for photosynthetic assimilates (Aguirrezabal et al. 1993) that will potentially vary depending on the intensity and location of the trophic competition (above - belowground) between the apple and the others plants of the system, here walnut and lucerne. Therefore, our initial objectives were to decipher the respective effects on the apple architecture development during its second year of growth, of competitions for light with walnut, and for soil nutrient and water with both walnut and lucerne. However, due to technical problems, the root growth survey could not be realized satisfactorily all along the season and it was decided not to include this topic in my dissertation (see appendix I for description of the *in situ* rhizotron experiment). Therefore, in this dissertation I will only present and discuss my works on the aboveground development of apple.

My objective was to study the effects of the distance between apple trees and walnuts, inducing presumably a gradient of competition, on various architectural and morphological aboveground features of the apple trees. More precisely, I analysed,

- the **shoot architecture** (topological distribution of lateral bud types) and the **geometry** of the previous-year growth, i.e. first annual growth, of the trunk (2016),
- the **growth dynamics** (length and number of leaves) of the trunk in the second-year of growth (2017),
- the **morphology of the leaves** (area and Specific Leaf Area).

This document starts by presenting the context and the literature in relation to our work before announcing the scientific hypothesis that are made. The second part is dedicated to presenting the materials and methods used. Finally, the third part presents our results that are discussed in a fourth part.

## I. Context

### I.1 Agroforestry: characteristics and stakes

Agroforestry has been present in European landscape since bronze Age (2500 BC) (Eichhorn et al. 2006). With the revolution of agricultural practices after the Second World War, agroforestry was soon abandoned and agriculture and forestry became distinct by their practices and their place in the landscape but also the institutions (Eichhorn et al. 2006). But agroforestry came back in the spotlight recently since it is seen as a possible answer to the environmental stakes that are facing agriculture. It remains a marginal agrosystem even if it is spread all over Europe from the Spanish “dehesa” to the meadow-orchard of north Europe (Eichhorn et al. 2006).

The World Agroforestry Centre defines agroforestry as so (Nair 1993):

*« A land-use system in which woody perennials (trees, shrubs, palms, bamboos) are deliberately used on the same land management unit as agricultural crops (woody or not), animals or both, either in some form of spatial arrangement or temporal sequence. In agroforestry systems there are both ecological and economic interactions between the different components. »*

This definition was then reworked by (Leakey 1996), whom introduced the landscape scale, and by (Noordwijk et al. 2016) whom gives a simpler and larger definition:

*« Agroforestry, a contraction of the terms agriculture and forestry, is land use that combines aspects of both, including the agricultural use of trees. »*

One of the hindrances identified to the adoption of agroforestry practices is the lack of knowledge (Gliessman 1985). Researchers took interest in these agrosystems only recently and studied a short part of their life span. Furthermore, these systems are hard to apprehend looking at the numerous trophic and ecological interactions that takes place.

We will first take a look at the different interactions that takes places in a temperate agroforestry system before looking at what mechanisms influence apple trees aerial growth.

#### **I.1.1 Interspecific interactions in a temperate agroforestry system**

Agroforestry systems, like other multi-species systems, are agrosystems designed to maximise resources (light, water, nutrients) usage in time and space by maximising positive interactions and minimising negative interactions (Jose et al. 2004). Therefore, using different indicators such as the land equivalent ratio (LER), it is possible to compare mono-specific agrosystems and multi-specific ones. Nevertheless, if we aim to achieve higher yields in an agroforestry system we need to understand what interactions take place and how they will influence plants growth. These interactions can be apprehended by using different types of interactions as defined by ecology (Table 1).



Table 1 : Different possible interactions between two species commonly described in ecology literature (Jose et al. 2004)

Interaction	Interaction's effect <sup>1</sup>		Nature of the interaction	Example in agroforestry
	Species 1	species 2		
Amensalism	–	0	One species is inhibited and the other one is unaffected	Allelopathy
Commensalism	+	0	One species is benefited and the other one is unaffected	Improved fallows
Competition	–	–	Both species are negatively affected as a result of each other's use of growth resources	Poorly managed alley cropping
Mutualism	+	+	Both species are positively impacted.	Mycorrhizae, <i>Rhizobium</i>
Neutralism	0	0	Neither species affects the other	Scattered trees
Predation, parasitism	+	–	One species benefits at the expense of another	Pest and diseases

<sup>1</sup>0 = no effect ; + = positive effect ; – = negative effect

It is possible to achieve higher yields in an agroforestry system if the interspecific competition is lower than intraspecific competition (Gliessman 1985). However, the relations between the different components of the agrosystems will be modified as the plants are aging. Perennial will grow and become more and more competitive for the annuals. To apprehend these complex systems, we can compartment interactions in aboveground and belowground interactions.

#### 1.1.1.1 Aboveground interactions in agroforestry

Interactions in agroforestry systems depend of the species or the disposition of the trees. The interactions intensity will be different if the trees are on the border of the field or inter-cropped (Jose et al. 2004). The most noticeable aboveground interaction is the competition for light between the species (Jose et al. 2004). When the young perennial is still shorter than the annual crop there can be competition but after two or three years the perennial will dominate the annual crop and intercept solar radiations impacting negatively the annual crop. Still, this interaction can be limited by diminishing the density of trees, their disposition or precocity (Chirko et al. 1996). However, the shade provided by the trees can also benefit the annual crops by limiting thermal stress (Quinkenstein et al. 2009; Lin 2007; Lin 2011).

Trees will also impact the microclimate that can benefit the shaded plants depending on the climate. For example, the row of trees can act as a windbreaker that will affect the evapotranspiration demand and therefore improve the water use efficiency (Quinkenstein et al. 2009). But a humid microclimate is also going to favour cryptogamic diseases (Gliessman 1985) which can be a problem for apple trees because of *Venturia inaequalis* the pathogen responsible for apple scab.

The introduction of trees will create new ecological niches by modifying the landscape that can offer new habitat that can contribute to increase the number and the diversification of auxiliaries/natural enemies and pest (Jose et al. 2004; Quinkenstein et al. 2009). Above ground interaction are summed up in Table 2.

Table 2: Above ground interactions between the trees and the annual crops in an agroforestry system: black = no impact ; red = negative impact ; green = positive impact ; arrows indicate the direction of the interaction (Jose et al. 2004; Jose et al. 2006; Ong et al. 1991; Forey 2016)

<b>Mechanism</b>	<b>Interaction(s)</b>	<b>Effect(s)</b>	<b>Interaction</b>
Shade	Competition	Both species intercept less sun radiation (young trees)	Trees ↔ Crops
	Amensalism	Species under the canopy will intercept less solar radiation (aged trees)	Trees → Crops
Wind and temperature buffering	Commensalism	Reducing wind speed and buffering temperatures (aged trees)	Trees → Crops
New ecological niche	Mutualism	Increases the number and diversity of auxiliaries	Trees ↔ Crops
	Predation, parasitism	Increases pest	Trees ↔ Crops

#### *1.1.1.2 Belowground interactions in agroforestry*

Belowground interactions between perennial and annual plants depend of the spatial location of their roots. In an ideal situation where the roots of each different species are present in different compartments of the soil, competition will be less important than where there are in the same (Gliessman 1985).

Perennial plants usually have the majority of their fine roots in the first thirty centimetres of the soil and so are in competition with the annual crop (Jose et al. 2006). However, most of the trees used in agroforestry have deep roots that will explore, if the depth of soil allows it, horizons of soils inaccessible to the annual plants (Rowe et al. 1998; Jose et al. 2001). Thereby, tree's roots can act as an interception net for the leached nutrients (Allen et al. 2004). These nutrients will then be available to the annual plants after decomposition of the litter in the case of deciduous trees and if the leaves are left on the plot. In the same way, trees will also be able to absorb nutrients coming from the bedrock alteration (Schroth 1995).

Deep roots could also act as a hydraulic lift if the top horizons are dryer than the bottom (Caldwell et al. 1998; Jose et al. 2004). If the quantity of water moved by this phenomenon is important enough, it could limit competition for water in mixed species systems. Even small amount can have a positive impact such as (i) making available nutrients that are not in a dry soil, (ii) facilitate root exploration and (iii) keeping roots active in dry soils and allow a quick recovery of activity (Pierret et al. 2016). Furthermore, roots exploration can be improved thanks to the pores created by the tree's roots on one hand and biological activity improving soil structure on the other hand (Hulugalle & Lal 1986).

Usually, trees roots occupy every soil horizons and thus are in competition with other plants for water and nutrients when they become a limiting factor. Even if this interaction can favour the separation of root systems (Pierret et al. 2016), yield will be negatively impacted (Smith et

al. 1999) as the trees development especially when they are still young (Parker & Meyer 1996). Some species will also exudate allelochemicals in the rhizosphere that can harm the annual crop (Rizvi et al. 1999). This is particularly true in our design as walnut tree exudate juglone which has long been recognised as the principal chemical responsible for walnut allelopathy (Jose et al. 2004). Belowground interaction are summed up in Table 3.

Table 3: Belowground interactions between the trees and the annual crops in an agroforestry system: black = no impact ; red = negative impact ; green = positive impact ; arrows indicate the direction of the interaction (Jose et al. 2004; Jose et al. 2006; Ong et al. 1991; Forey 2016)

<b>Mechanism</b>	<b>Interaction(s)</b>	<b>Effect(s)</b>	<b>Interaction</b>
Root absorption	Competition	For water and nutrients in the top soil where the root of both species are present	Trees ↔ Crops
	Commensalism	The trees can act as a hydraulic and nutrient lift for the crop, and the crop fertilisation can profit the trees (interception net)	Trees → Crops Trees ← Crops
Root exudates and decomposition	Commensalism	Improvement of soil carbon and nutrients Soil fauna stimulated	Trees → Crops Trees ← Crops Trees → Crops
	Mutualism	Mycorrhizal bridge	Trees ↔ Crops
	Predation, parasitism	Inhibiting growth through allelochemicals exudation	Trees → Crops Trees ← Crops
Hydraulic lift and nutrient pump	Commensalism	In dry soils, deep water soil is lifted up by the tree's roots. Leached nutrients are recycled through litter mineralisation	Trees → Crops
New ecological niche	Mutualism	Increases the number and diversity of auxiliaries (nematode predating on bacterium and fungal)	Trees ↔ Crops
	Predation, parasitism	Increase in pest (phytophagous nematodes)	Trees ↔ Crops

All these interactions will impact the development of the different species in the agroforestry system. In our experimental design, the walnut trees are already aged so they won't be much impacted by the presence of the apple trees but will be highly competitive for light, water and nutrients. The apple trees have to grow in environmental conditions that are radically different than in traditional orchards. It is interesting to analyse the aerial growth of the apple trees so we can understand the consequences of such conditions on their development.

## I.2 The Apple tree: influence of aboveground environment on growth and ramification/branching

### I.2.1 **The apple tree (*Malus domestica* Borkh.)**

Apples are among the oldest and most important fruit crops in the world (Harris et al. 2002). They have been cultivated since ancient times, in fact, archaeological studies have shown that they were cultivated already in 1000 BC (Juniper et al. 1998).

Cultivated apples are a result of extensive ancient hybridization of various species of the genus *Malus* Mill., a member of the *Rosaceae* Juss. family, subfamily *Pomoideae* (pome fruits) (Jackson 2003; Webster 2005). Over hundred botanical names have been published for the cultivated apple (Qian et al. 2010), however, *Malus domestica* Borkh. is now the correct binomial nomenclature for the cultivated apple (Qian et al. 2010). Some morphological characteristics shared by apple cultivars in the world are: woolly pubescence on young stems and on the lower surface of the leaves, dull green leaves, elliptic-ovate in shape, with irregularly saw toothed margins, woolly pubescence on flower stalks and calyx, and pome fruits indented at the base with persistent calyx (Webster 2005).

### I.2.2 **Apple tree morphology**

#### I.2.2.1 *Shoots and buds*

Nowadays, apple trees are almost exclusively compound trees consisting of a scion grafted on a rootstock (Jackson 2003; Webster et al. 2005). Rootstocks are used to avoid juvenility, to control vegetative growth, to promote flower-bud formation, to improve cropping efficiency and quality of the fruits, and in some cases to provide winter hardiness and provide resistance or tolerance to some telluric diseases (Hanke et al. 2007; Webster et al. 2005).

The scion is the productive part of the tree that bears the different buds and three different type of shoots: (i) extension, (ii) non-extension and (iii) bourse shoots. Extension shoots are long and indeterminate in growth. Non-extension shoots are shorter, determinate in growth (Webster et al. 2005). Non-extension shoots can be classified depending on their length: (i) spurs (very short shoots), (ii) dards (5-10 centimetres) and (iii) brindles (10-20 centimetres). Bourse shoots originate from vegetative primordia at the axil of bourse leaves in spurs and may become extension shoots or remain short as bourse shoots (Jackson 2003; Webster et al. 2005).

All shoots emerge from buds which have the potential to produce leaf primordia only or both leaf and flower primordia. If a bud produces leaf primordia only, it is considered a vegetative bud. On the contrary, if a bud produces flowers in addition to leaf primordia, it is considered a mixed (flower) bud (Figure 1). Flower buds are found terminally on all types of shoots and terminally or axillary on

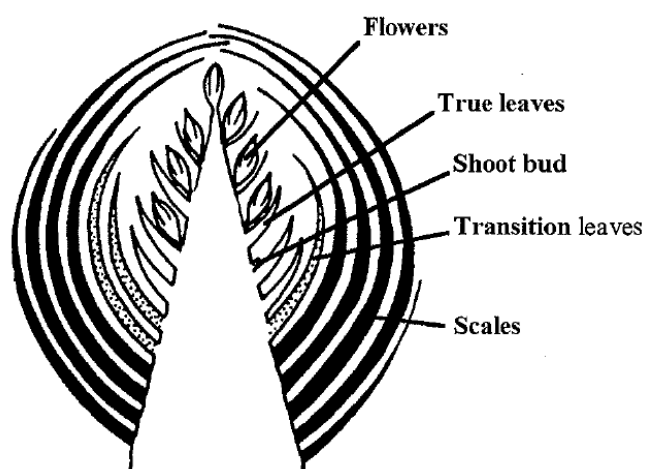


Figure 1: Apple flower (fruit) bud in diagrammatic longitudinal section, showing foliar appendages and flower buds (Abbot 1970).

extension shoots after vegetative growth has stopped (Jackson 2003; Webster et al. 2005).

#### *1.2.2.2 Annual growth cycle*

Apple trees are adapted to temperate climate in which they overcome large seasonal changes in environmental conditions. Such adaptation is the result of an annual growth cycle in which all developmental processes are synchronised with the annual course of the growing conditions (Hänninen & Kramer 2007). The growth cycle of an apple tree lasts for approximately one year from budburst, flowering, shoot growth, fruit set and development, to shoot growth cessation, flower bud formation, leaf abscission and winter dormancy.

Early in the spring a high proportion of buds emerges from dormancy after fulfilling their chilling and heat requirements (Luedeling 2012). There is a genotypic variation on both the amount of chilling required to break dormancy and the threshold temperature for bud-break and generally, in cultivars with a low chilling requirement, bud-break occurs at lower temperatures (Faust 1989; Jackson 2003; Webster et al. 2005).

Flower buds develop flower clusters that pass through a series of phenological stages. Vegetative buds produce a rosette of leaves with a “naked bud” in the centre (a bud without bud scales), or grow out to form a new shoot (Webster et al. 2005). New shoots continue to extend during the summer. By the end of the summer, extension growth stops in these shoots, and is followed by the formation of a terminal resting bud (Webster et al. 2005). From this point onwards, flower buds may be formed in terminal or axillary buds within the current year’s extension shoot.

Shoot growth and formation of flower buds are processes that occur in parallel to fruit development. Consequently, a strong competition for immediate available resources takes place between these developmental processes during summer (Hanke et al. 2007). It has been reported that the presence of fruit has a negative effect on shoot growth (Jackson 2003), mainly due to the strong sink effect of fruits (Webster et al. 2005). Inhibition of flower-bud formation by fruits has also been reported and explained as a result of the inhibitory effect that hormones from developing seeds impose on this process (Jackson 2003).

As autumn progresses, temperature and day length decreases, triggering a series of physiological changes in the trees that lead to leaf abscission and development of the endodormancy. Changes such as chlorophyll degradation, which causes decolouration of leaves, remobilization of leaf components to the woody parts of the tree, followed by degradation of cell walls causes leaves shedding (Faust 1989). By the time of leaf abscission, the tree is in a state of rest in which bud-break does not occur even if environmental conditions are conducive to do so (Jackson 2003).

### **1.2.3 Abiotic factors influencing apple tree development**

The growth of woody plants has two components: increment of biomass and the architectural arrangement of this biomass in space. The first is due to the physiological processes that lead to net carbon, water and nutrient uptake, and the second is a result of branching patterns (Küppers 1989). Correlations between growth habit and environment have long been known (Slavík 1981), indicating that particular architectures and their fundamental branching patterns are advantageous in certain habitats. We will review how aboveground competition can influence the trees development.

Aboveground competition involves a single resource, light, and it is the light environment that primarily limits potential benefits and that demands specific canopy shapes (Küppers 1989). Higher plants not only transform solar energy into chemical energy through the process of photosynthesis but also use light as an informational signal to control a multitude of physiological responses throughout their life cycle. The light quality (spectral composition), quantity, direction, and duration change depending on the season, latitude (magnitude of day-length variable), and local condition (weather, position within plant communities). For instance, light under a plant canopy has a typical signature with a strong

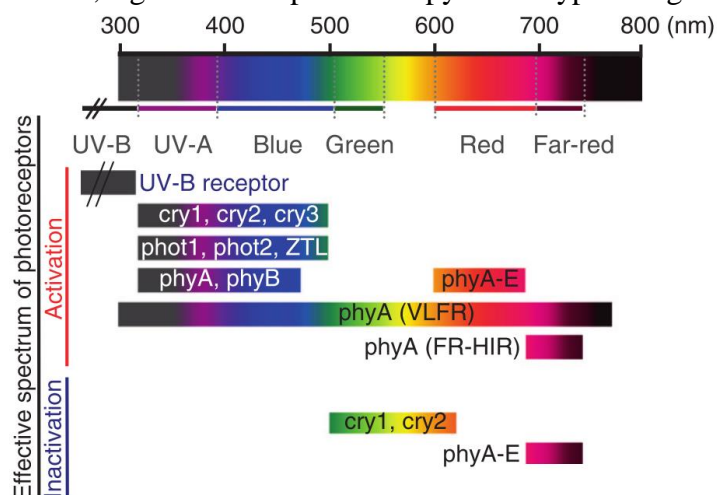


Figure 2: Effective spectrum of photoreceptors for activation and inactivation from (Kami et al. 2010).

reduction of blue and red light absorbed by the photosynthetic pigments while levels of green and in particular far red (FR) light (near infra-red  $\lambda = 700-750$  nm) remain relatively high (Kami et al. 2010). To sense such a diversity of light conditions, higher plants possess multiple light sensors that can be activated or inactivated depending on the light quality (Chen et al. 2004). Four classes of photoreceptors have been identified in Arabidopsis. These photoreceptor families are present in all sampled higher plants although the number of members in each family is somewhat variable (Figure 2).

Depending on the photoreceptors that are activated different physiological response will be observed. Regarding vegetative growth light will affect a diversity of physiological traits as leaf expansion, movement, positioning, stomata development and opening, chloroplast accumulation, petiole growth, stem and internode elongation and even root development (Kami et al. 2010).

In a shaded environment where light capture is essential we can expect longer stem and internodes (Kami et al. 2010), leaves with high specific leaf area (SLA) (Lambers & Poorter 1992), increase in leaf area and reductions in root/shoot ratio (Grime 1977). However, these phenotypic adaptation are most apparent in competitive species characteristic of unshaded or lightly shaded environment (Grime 1977). Shade tolerant species tend to grow slowly and to show little morphogenetic response to shade treatment (Grime 1977).

### I.3 Scientific question

Agroforestry systems raise numerous questions that require to thoroughly study them at a scientific level as well as about their agronomic potential. It's to answer to these questions that the UMR System created in spring 2016 an experimental design consisting of forestry trees (23-year-old walnut trees), fruit trees (2-year-old apple trees) and an herbaceous part (5-year-old lucerne).

The scientific question we are trying to answer in this report is not so much as if there are differences in aerial growth between apple trees in an agroforestry system and apple trees in a traditional orchard as to where these differences are located.

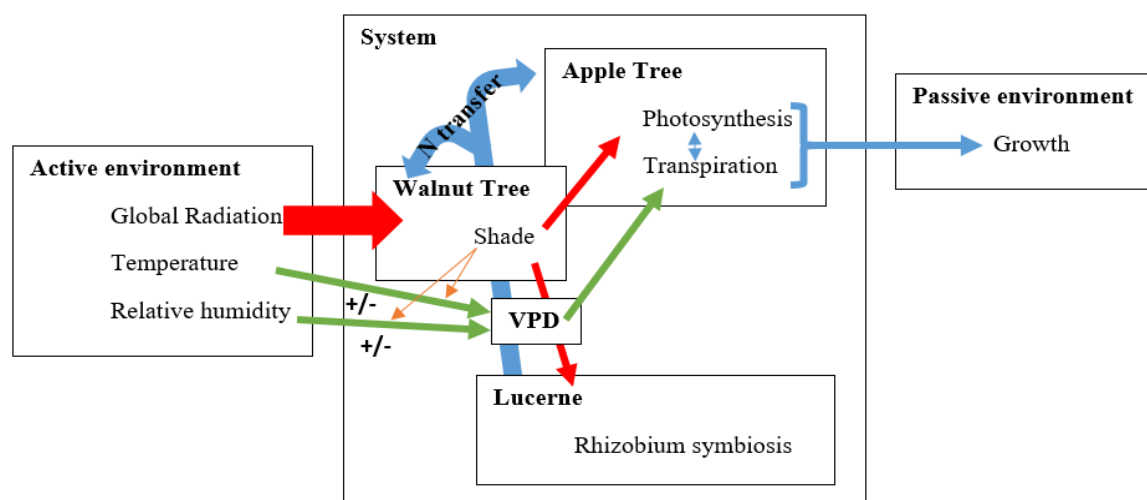


Figure 3: Concept map of the aerial interactions in the agroforestry system studied.

After realising a concept map (Figure 3) where we considered that the intensity of the interactions in the agroforestry system can be correlated to the distance between the plants and especially the distance to the walnut trees that, we suppose, will be responsible for most of the aerial interactions we made two hypotheses,

- the aerial environment differs in a mature agroforestry system (aged walnut tree) compared to a traditional orchard,
- the aerial interactions (microclimate and especially shade) can influence the apple tree growth (etiolation ...) and ramification/branching,

NB: the possible effects of belowground competitions could not be explored satisfactorily in this 6-month experiment.

To make sure that the differences we would possibly observe were due to the aboveground interactions in the agroforestry system we made sure to reduce all limiting factors as well as we could. Therefore the apple trees were irrigated and fertilised and we avoided pests and disease that could influence aerial growth.

We divided our apple trees in two blocks to take into account the heterogeneity of the plot and three modalities consisting of the agricultural control (AC), the agroforestry inter-row (AF\_IR) as to between the rows of walnut trees and agroforestry row (AF\_R) when the apple trees were planted on the same row as the walnut trees.

## II. Materials and methods

### II.1 Description of the field experiment

#### II.1.1 Experimental design of the plot A1 of “domaine de Restinclières”

The “domaine de Restinclières” is located in the south-east of France fifteen kilometres north of Montpellier (Figure 4). It’s a regional domain of 220 ha with 54 ha of arable lands where an agroforestry experimentation was started twenty three years ago. The agroforestry layout chosen in the 90’s was conceived taking in account modern agricultural practices.

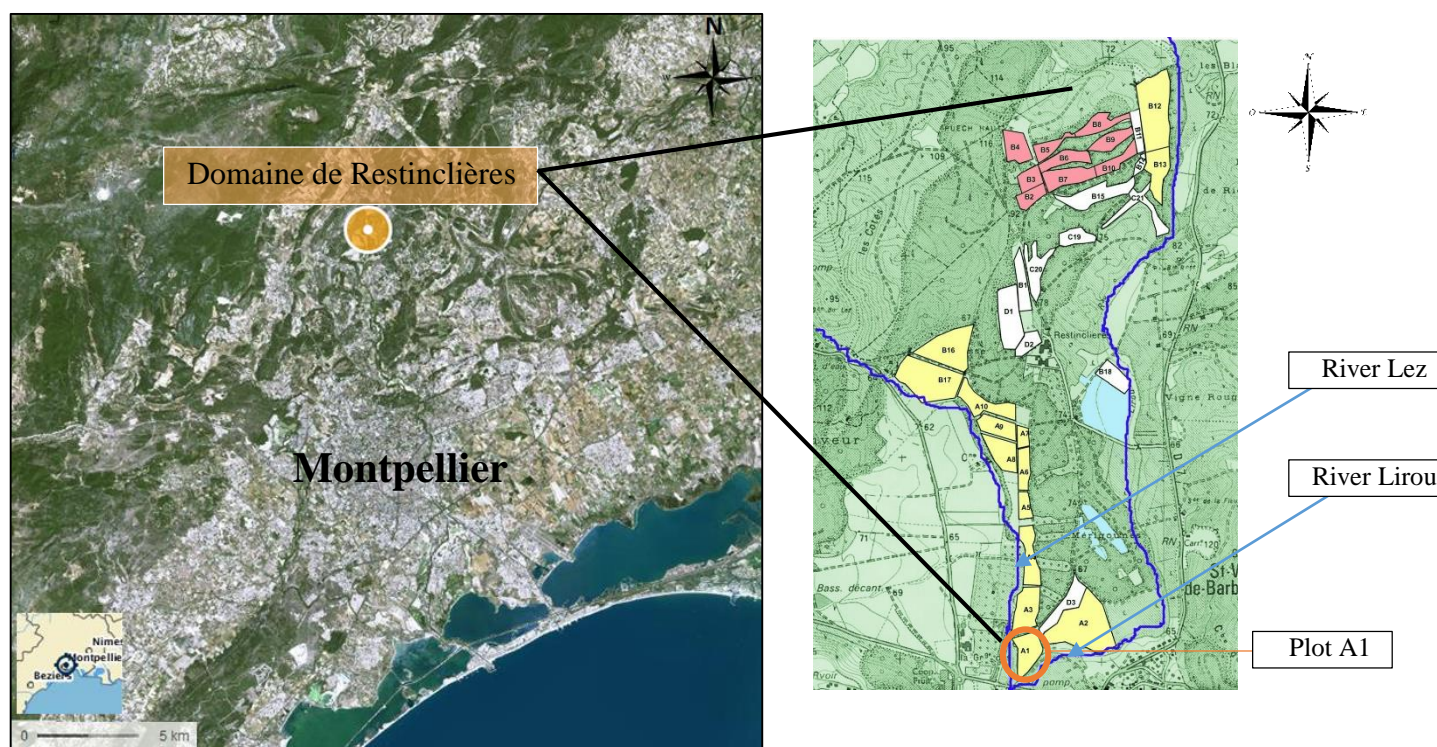


Figure 4: Geographic localisation of the “domaine de Restinclières” and the plot on which is located the apple – agroforestry experiment (source: googlemaps, UMR System)

Therefore, the rows of forestry trees were planted to let the combine harvester pass between tree rows. Different forestry trees, whose wood is precious as *Juglans* (walnut tree) or *Sorbus domestica* (sorb tree), were planted in association with other perennials (vine) or annual crops (wheat, barley, rape) (Fournier 2002).

The lands are cultivated by a farmer who works with the research teams. Plot A1 was selected for the installation of the experiment because it is the only one that is not maintained or cultivated by the farmer. Therefore it was possible to plant apple trees and manage them as we wanted without any external disturbances. The walnut trees were planted in 1995 with a distance of thirteen metres between the rows and 4 metres on the row. In 2004, there was a thinning, half of the trees, unevenly distributed in the plot, were eliminated.

The experimental design is composed of two blocks in which we have the three modalities comprising 83 and 65 apple trees, respectively (Table 4).



Table 4: Number of apple trees in each block per modality. AC: Agricultural control; AF\_IR: Agroforestry inter-rows; AF\_R: Agroforestry rows.

Block	Block 1			Block 2		
Modality	AC	AF_IR	AF_R	AC	AF_IR	AF_R
Nb. Trees	18	40	25	8	45	12
Nb. Modality	83			65		
Nb. total	148					

The rows of walnut trees are oriented west to east (Figure 5). Block 1 is located in the north part of A1 plot near a hedge and block 2 is in the south west of the plot near the Lez. The

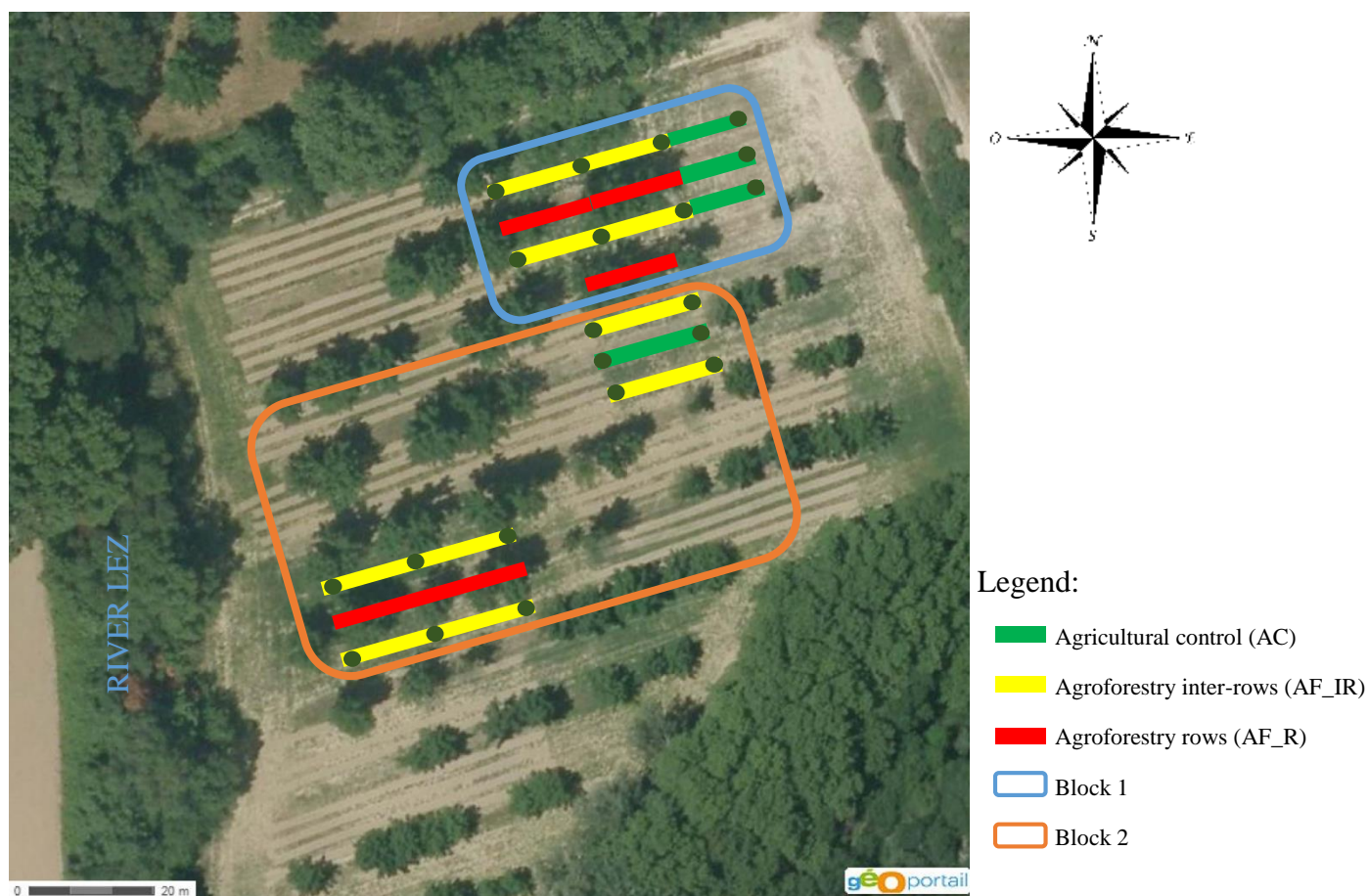


Figure 5: Representation of the apple trees experimental design of domaine de Restinclières.

agricultural controls are located as far of the walnut trees as possible. The apple trees on the inter-row are at 6.5 metres of the rows. Distances between apple trees, and between apple trees and walnut trees, on the row, are 1.3 metres which is commonly found in traditional high density apple orchards (Robinson 2003).

### II.1.2 Plant matériel and plot management

There are three different plant in our plot, the forestry trees (hybrid walnuts *Juglans regia x nigra*), the apple trees (Dalinette cultivar grafted on G202 rootstock) and the lucerne as grass cover except in walnut, apple and walnut-apple row.

The apple trees are managed according to the organic technical specifications following advice from the CETA Hérault-Vidourle. Therefore, a cultivar that is resistant to apple scab,

the disease responsible most of the treatments, was necessary. That is the case of Dalinette which is a vigorous cultivar that is recommended in organic orchards. G202 rootstock is a semi-dwarfing rootstock. It is fire blight and phytophthora resistant, but also has good resistance to woolly apple aphids, which is an important rootstock pest in many warmer climates. These cultivar and rootstock were selected because of the distinctive design and management strategy. In an agroforestry system there are much more interaction and competition. Thus, we needed an apple tree that would be suitable to this particular environment.

So far, the apple trees were being irrigated with a double drip system of 1.6 L/hour and were given the equivalent of the evapotranspiration over a week as advised by the technician from the agriculture chamber. The apple trees were given 12g/tree of iron-chelate two weeks after bud burst and the equivalent of 32 kg N/ha in two times through the irrigation system. It was also necessary to treat the trees against aphids once using Supreme (none organic) at full dose. The only thinning intervention that was made is to eliminate all ramification below the first wire (around 60 cm from the soil surface).

The lucerne, that was already 5-year-old, was left on the inter-row because, as a leguminous plant, it will be less competitive for nitrogen, thanks to the Rhizobium symbiosis, and can even transfer N to neighbouring plants (Pirhofer-Walzl et al. 2012). The lucerne is harvested and left on the plot three to four times during the season before it has gone to seed.

Weeds on the row were controlled using dry lucerne and ramial chipped wood (RCW).

### II.1.3 Soil and climate conditions

#### II.1.3.1 Mediterranean climate

The plot is situated in the Mediterranean region which is known for its typical climate whose name is the same even if it is also encountered in other region of the globe. The Mediterranean climate is usually characterised by rainy winters and dry, hot summers. As the “domaine de Restinclières” has been an experimental site for agroforestry since 1995, it was possible to obtain the data from a weather station on site (Figure 6).

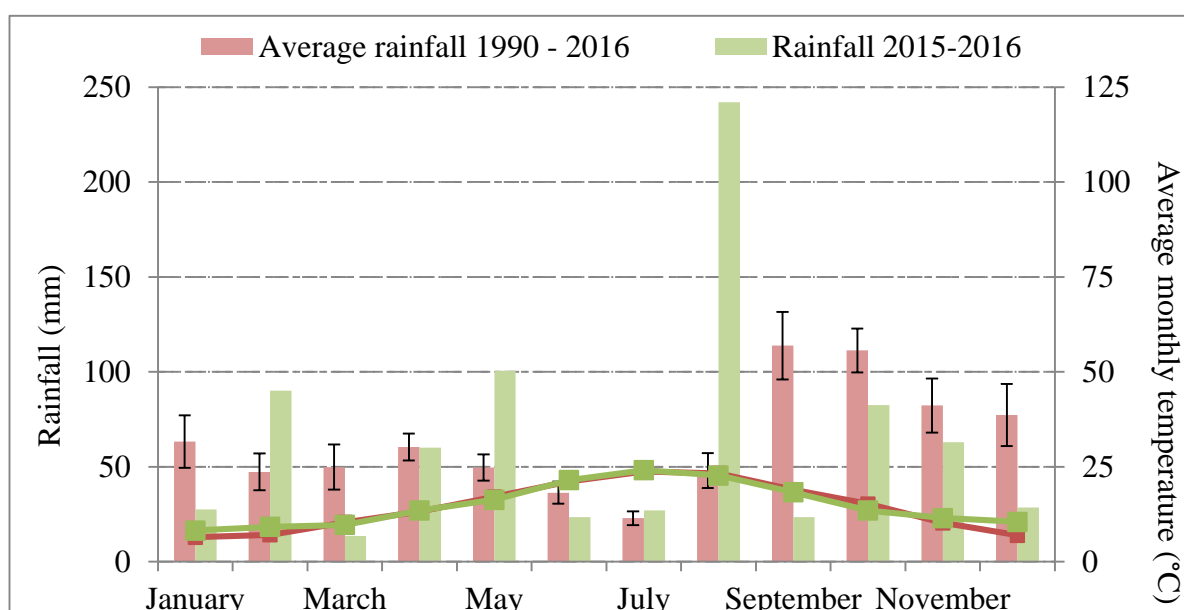


Figure 6: Average rainfall/temperature diagram based on the data from the weather station on plot A2 from domaine de Restinclière experiment site.

Under the Köppen climate classification, "hot dry-summer" climates (classified as *Csa*) and "cool dry-summer climates (classified as *Csb*) are often referred to as "mediterranean". The "C" stands for temperate climate with an average temperature between 0 and 18°C. The second letter indicates the precipitation pattern, "s" represents dry summers. The third letter indicates the degree of summer heat with "a" meaning that the average temperature in the warmest month is above 22°C and "b" below 22°C.

It is also important to note that the climate is highly variable during the year but also from one year to the other. As we can see on the diagram there is usually a drought period from June to August. However, due to the high variability of the climate it is hard to predict what the precipitation pattern will look like. In August 2016 for example, there was over 200 mm of rain leading to a drought in September with only 25 mm of rainfall over the all month. Therefore, it is an obligation to water the orchards under Mediterranean climate during spring and summer.

### II.1.3.2 Plot A1 soil characteristic

According to the technicians in charge of looking after the different experiment taking place at the domaine de Restinclières there is a gradient in soil depth on the plot. Soil is deeper in south part near the rivers that in the north part near the paths.

Thanks to a previous study on this plot we have a soil analysis at different depth and different distances from the walnut trees from 2013 at our disposal (Table 5).

Table 5: Soil analysis of plot A1 from "domaine de Restinclières" done in 2013

Dstce Walnut (m)	Depth (cm)	Clay <sup>1</sup> (g/kg)	Fine silt <sup>2</sup> (g/kg)	Coarse silt <sup>3</sup> (g/kg)	Fine sand <sup>4</sup> (g/kg)	Coarse sand <sup>5</sup> (g/kg)	Soil moisture (%)	pH	C (g/kg DS*)	NO3 (mg/kg DS*)	NH4 (mg/kg DS*)
0	0-20	272	240	150	240	96	8,1	8,1	12,9	12,9	0,2
0	20-40	274	221	188	224	94	7	8,4	6,3	2,6	0,3
0	40-60	264	215	228	218	76	7	8,4	5,6	1,4	0,5
0	60-100	284	277	199	134	106	8,4	8,7	4,8	0,4	0,6
0	100-150	418	410	132	28	12	12,8	8,6	4,5	0,1	0,4
0	150-200	436	442	81	26	16	9,6	8,6	4,2	0	0,3
2	0-20	277	237	174	214	98	7,9	8,5	6,9	4,4	0
2	20-40	261	213	204	206	116	9,1	8,5	7	3,8	0,1
2	40-60	309	315	191	150	36	7,7	8,4	6,5	4	0,3
2	60-100	228	331	185	180	76	10,3	8,5	4,9	0,6	0,4
2	100-150	309	482	154	48	7	9,3	8,5	4,6	0	0,1
2	150-200	414	431	116	33	7	9,3	8,5	4,7	0	0
4	0-20	197	254	181	268	99	10,5	8,4	7,9	5	0
4	20-40	165	257	182	284	112	8,9	8,5	6,2	3,9	0
4	40-60	187	272	194	252	95	7,3	8,6	5,2	1,3	0,2
4	60-100	243	318	161	176	103	6,9	8,6	4,6	0,1	0,2
4	100-150						9,2	8,6	4,8	0	0
4	150-200	344	517	108	24	7	10,7	8,6	5,1	0	0,1

<sup>1</sup><2µm ; <sup>2</sup>2 to 20µm ; <sup>3</sup>20 to 50µm ; <sup>4</sup>50 to 200µm ; <sup>5</sup>200 to 2000µm ; \*Dry soil

The soil texture varies with the depth but not with the distance to the tree. The proportion of fine particles (clay and silt) increases with depth resulting in the soil texture evolving from a “clay loam” to a “silty clay” (Figure 7). This is not surprising as smaller particles tend to leach easier than bigger particles. As we do not know the nature of the clay particles, we can’t conclude on the soil property. However, a loamy top soil has a medium infiltration rate, and a moderate plant-available water-holding capacity. Root growth is not restricted but can be susceptible to compaction and water erosion (Moody & Cong 2008).

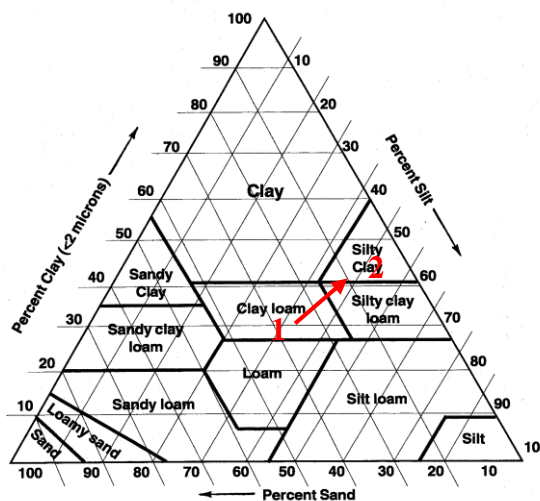


Figure 7: Representation of the texture evolution with depth of plot A1 soil from the “domaine de Restinclières” on a soil texture triangle.

The soil pH between 8.1 and 8.6 is regarded as an alkaline soil dominated by Na, Ca and Mg carbonates (Moody & Cong 2008). Micronutrient deficiencies may be present because Zn, Fe and Mn become less available as the pH increases, whereas MO becomes more available (Moody & Cong 2008).

The soil organic content is higher next to the trees probably thanks to the litter of leaves and root decomposition. But still, the percentage of organic matter is still in the average (2.2%) of French soils (Meersmans et al. 2012) next to the trees and very low (<1.5%) 2 and 4 metres away.

Looking to nitrogen, there is almost no ammonia and very little nitrate. There is a difference in nitrate concentration depending on the distance to the walnut trees where the higher concentration are close to trees. This is probably the result of the organic matter (from the roots and litter) mineralisation therefore assessing the role of the tree in the cycle of nutrients. There is no nitrogen (ammonia or nitrate), or very little, under 150 centimetres. This is probably because most of the nutrients are leached in autumn and winter because of the water table rising up.

## II.2 Characterisation of aboveground environment

### II.2.1 Microclimate

To highlight microclimates differences in our different modalities we also installed, at the beginning of July 2017, pyranometer (SP1110 made by Campbell Scientific™) to measure the global radiation and humidity/temperature probes (Vaisala HUMICAP® HMP155 and HMP45C) in weatherproof housing. The sensors



Photograph 1: SP1110 pyranometer (left) and HMP155 in weatherproof housing (right).

recorded global radiation air temperature and humidity every hour (Photograph 1).

## II.2.2 Incoming light

To characterise the environment of our apple trees we processed images taken with a fisheye lens. We took pictures before and after walnut trees bud burst to see how evolved the difference in light for apple trees throughout the growing season.

We used a fisheye lens and camera provided by Regent Instruments Inc. (Photograph 2).



Photograph 2: 180° fisheye lens (left) and Sony camera (middle) used to take hemispheric photographs (right). The camera and lens were provided and calibrated by Regent Instruments Inc.

Ten pictures per modality were taken next to the apple tree at pre-dawn to avoid direct sun light. The photographs were then threshold in black and white using PiafPhoem before being processed in PiafLA to obtain the uniform overcast sky (UOC) and standard overcast sky (SOC) values. The difference between these two models is that in uniform overcast sky (UOC) model incoming diffuse radiation is the same from all sky directions whereas in the standard overcast sky (SOC) diffuse model, diffuse radiation flux varies with zenith angle (Fu & Rich 1999).

The diffuse radiation under the canopy is calculated for each pixel of the photograph. Each pixel is given a value depending on his colour (white or black). This value is then weighted by is the space it occupies on the sky (solid angle) and the distribution of incoming radiation (stronger at the zenith than the horizon) depending on the model used (UOC or SOC).

According to PiafLA resource here is how the value of a pixel is calculated:

$$UOC_{pixel} = \frac{SolidAngle_{pixel}}{\pi} \times \cos ZenithalAngle_{pixel} \times Colour_{pixel}$$

$$SOC_{pixel} = \frac{3 \times (1 + 2 \sin(\pi - ZenithalAngle_{pixel}) \times \cos(ZenithalAngle_{pixel})) \times SolidAngle_{pixel}}{\pi} \times Colour_{pixel}$$

With:

$$SolidAngle_{pixel} = \left(\frac{\pi}{rayon}\right)^2 \times \frac{\sin(ZenithalAngle_{pixel})}{ZenithalAngle_{pixel}}$$

$ZenithalAngle_{pixel}$ : pixel's height ( $\pi$ : horizon; 0: zenith)

$Colour_{pixel}$ : 1 if the pixel is white and 0 if it's black

## II.3 Apple trees characterisation

### II.3.1 **Apple tree water status: water potential at pre-dawn and midday**

We measured the water potential in the leaves of 15 trees per modality at pre-dawn, when the tree is at a balance with the soil and at midday when the hydric stress is supposed to be the higher. Irrigation was suspended for four days before the measurements.

Leaf water potential at pre-dawn (8<sup>th</sup> of august 2017) and midday (11<sup>th</sup> of august 2017) was measured on fully expanded leaves situated 7 nodes below the growing terminal apex of a long shoot. For water potential at pre-dawn, the leaves were detached and the measurement was done immediately. For the midday potential, the leaves were inserted into a plastic bag covered by an aluminium foil two hours before the measure. The bags were then clenched to the leaves petiole to prevent any contact with light or air (Photograph 3). Then the measures were done in the same way as for water potential at pre-dawn.



Photograph 3: Apple trees with the leaves for midday water potential inserted in the plastic bag covered in aluminium foil (left) and one of the two pressure chambers used for the measurement of water potential in the leaves (right).

The same trees were used for pre-dawn and midday water potential. Two intact leaves per tree were selected. Two pressure chambers were used simultaneously to complete each set of measurements rapidly to minimise the time elapsed between the first and the last measurement of each set. The average of two leaves was taken to represent each tree. One of the chambers could not go below -2.7 MPa so it's possible that we underestimated midday water potential. However, there was less than five measures that reached -2.7 MPa.

### II.3.2 **One year old shoots**

#### II.3.2.1 *Geometry: Slenderness and taper*

Before bud burst and looking at the shoot growth in 2017, we measured the length and diameter of the apple trees after one year using a measuring tape and a calliper. As the trunk is not perfectly round, we took the average of two measures done with the calliper (Figure 8). The diameter was measured on an internode at the base and the apex of the stem. We then considered an average diameter of the all trunk calculated using the following equation.

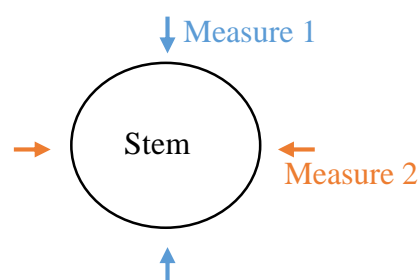


Figure 8: Schematic representation of the trunk diameter measurement methodology.

$$D_{stem} = \frac{\left(\frac{D_{base1} + D_{base2}}{2}\right) + \left(\frac{D_{apex1} + D_{apex2}}{2}\right)}{2}$$

With:

$D_{stem}$ : Diameter of the trunk;

$D_{baseX}$ : Diameter of the base of the trunk where x corresponds to the measurement;

$D_{apexX}$ : Diameter of the apex of the trunk where x corresponds to the measurement.

We used this data to calculate two indicators traditionally used in forestry: slenderness that corresponds to the ratio of the stem's length on the mean diameter, and taper that is the difference of diameter between the base and the apex divided by the trunk's length.

#### *II.3.2.2 Architecture: Buds type and topology*

Every week after bud burst we looked at the type of new buds that appeared and their relative position on the trunk. Buds were either noted as latent (L), or growing being vegetative (V), or flower (F), or extinct (E). In this latter case, the bud had stopped growing after budburst without making any leaves.

#### *II.3.2.3 Phenology: Flower buds*

We also followed the phenology of the flower buds every week. We used the Fleckinger chart to name the different flower stage.

### **II.3.3 2017's growth**

#### *II.3.3.1 Growth dynamics*

After the budburst, we followed the growth of the shoot in 2017 by measuring his length every week with a measuring tape and counted the number of leaves. Only the leaves longer than one centimetre were taken into account.

#### *II.3.3.2 Leaf area (LA) and specific leaf area (SLA)*

The specific leaf area was calculated for fifteen leaves of each modality using the following formula:

$$SLA = \frac{LeafArea}{DryMass}$$

The leaves were collected at pre-dawn on site and put into plastic bags with humid paper towel so they want dry up. The plastic bags were then in an ice compartment until we came bag to the laboratory.

The leaf area was measured using a scanner and the software WinFOLIA®. Once scanned the leaves were placed individually in a paper bag and placed in an oven at 60°C for 48 hours. Once dried each leaf was weighted on a scale with a precision of 0.01g.

## **II.4 Data analysis**

Statistical analyses of variance were performed for most of our collected data when the hypothesis of normal distribution and homoscedasticity of the residual were confirmed. If not, we used a non-parametric test, Kruskal-Wallis test by ranks (Chan & Walmsley 1997). The effect of the block was tested each time but was never significative.

On the data concerning geometry of the growth in 2016 we chose to make a regression between the length and the average diameter of the stem. We used the standardised major axis

(SMA) method for line fitting between our variables because it calculates the line of best fit without a priori hypothesis on the dependence of one variable on the other (Warton et al. 2006). First, the effect of the modality on the slope was tested with two alternatives, no difference in slopes or difference in slopes. In the first case, differences for the intercept and for shift along the lines with common slopes could then be carried out (Warton et al. 2006). In the second case, it indicated that the modality affected the relationship between the two variables, and the other tests were not relevant. We also looked if there was an allometric relationship between stem length and diameter. Allometric relationships usually refer to a power mathematical equation:  $Y = b.X^a$ , linearised as  $\log_{10}(Y) = \log_{10}(b) + a.\log_{10}(X)$  where ‘a’ is the slope and ‘ $\log_{10}(b)$ ’ the intercept. When  $a = 1$ , it indicates an isometric relationship. When  $a \neq 1$ , it denotes an allometric relationship, either negative when  $a < 1$  ( $Y/X$  decreases when  $X$  increases) or positive when  $a > 1$  ( $Y/X$  increases when  $X$  increases) (Pigliucci & Preston 2004).

To analyse the flower bud phenology we use an ordinal multinomial model because the response (phenological stage) can take more than two values. Furthermore the phenological stage are ordered ( $A > B > C > \dots > J$ ) and, as we are not making any assumptions on the distribution of the data, we used an ordered logit model (Faraway 2006).

Statistical analyses were done using RStudio® software with R version 3.4.0 and, with package ‘car’, ‘ggplot2’, ‘agricolae’, ‘MASS’, ‘nnet’, ‘foreign’. SMA correlation was done using the SMATR software (Standardised Major Axis Tests and Routines, <http://bio.mq.edu.au/ecology/SMATR/>, accessed 1 September 2012; (Warton & Wright 2006)). A significance level of  $p < 0.01$  was used for all the analyses.

### III. Results

#### III.1 Characterisation of aboveground environment

##### III.1.1 A microclimate generated by the presence of walnut trees?

We used data from the HMP sensors that recorded air temperature and air humidity from the first of July 2017 to the twentieth of August 2017 (Figure 9, Figure 10). For global radiation the data only started on the twelfth of July until the same day (Figure 11).

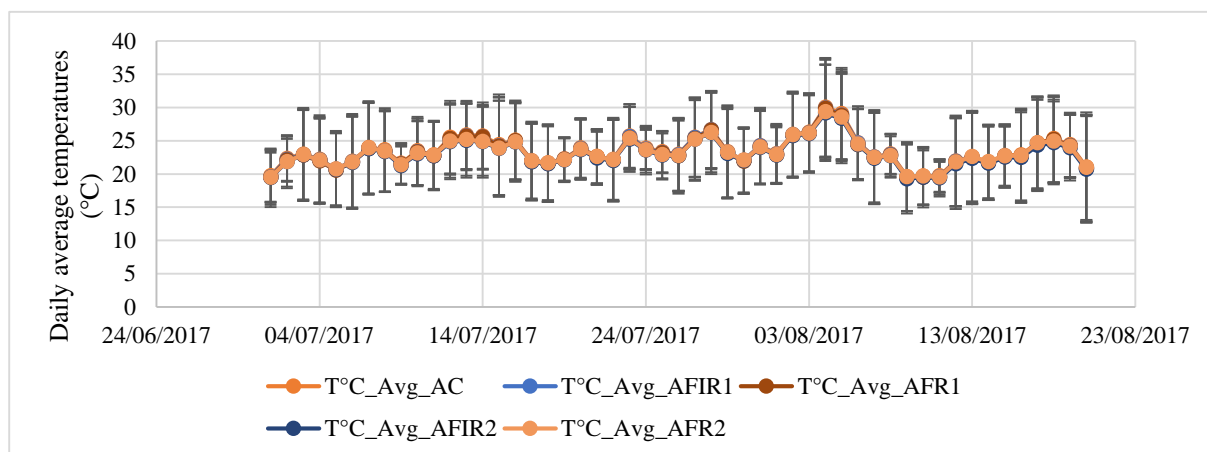


Figure 9: Daily average temperature (mean±SD) recorded by HMP155 sensors on the plot A1 from the domaine de Restinclières recorded from the 1<sup>st</sup> of July 2017 to the 20<sup>th</sup> of August 2017. There are 5 sensors on the plot, one the open is the control (AC) and the four others are in each agroforestry modality and in the 2 different blocks (AFIR1/AFR1; AFIR2/AFR2) with AFIR: Agroforestry inter-row and AFR: Agroforestry row.



No significant difference was observed for average temperature and relative humidity using a Kruskal-Wallis rank test.

There are significant differences looking at the global radiation (Figure 12).

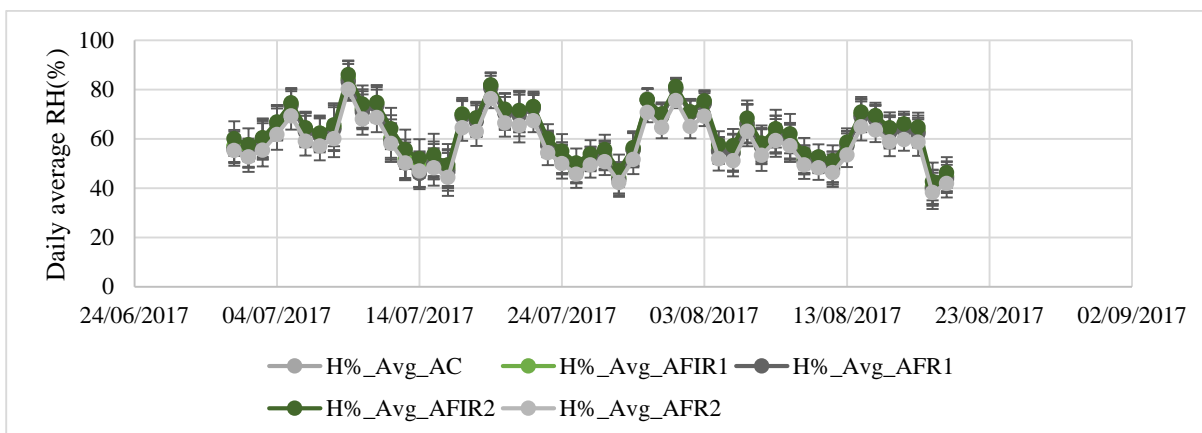


Figure 10: Daily average relative humidity (mean±SD) recorded by HMP155 sensors on the plot A1 from the domaine de Restinclières recorded from the 1<sup>st</sup> of July 2017 to the 20<sup>th</sup> of August 2017. There are 5 sensors on the plot, one the open is the control (AC) and the four others are in each agroforestry modality and in the 2 different blocks (AFIR1/AFR1; AFIR2/AFR2) with AFIR: Agroforestry inter-row and AFR: Agroforestry row.

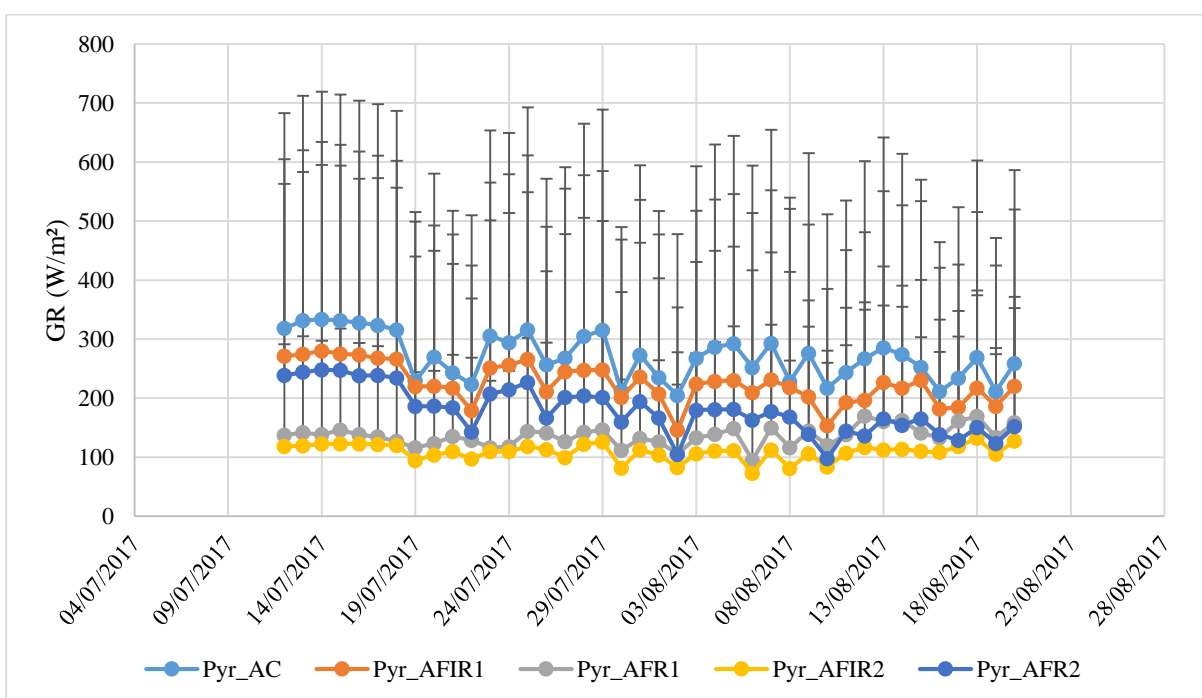


Figure 11: Daily average global radiation (mean±SD) recorded by SP1110 pyranometers on the plot A1 from the domaine de Restinclières recorded from the 12<sup>th</sup> of July 2017 to the 20<sup>th</sup> of August 2017. There are 5 pyranometers on the plot, one the open is the control (AC) and the four others are in each agroforestry modality and in the 2 different blocks (AFIR1/AFR1; AFIR2/AFR2) with AFIR: Agroforestry inter-row and AFR: Agroforestry row.

Each modality is different from the others as we have as much statistical group than modalities. The pyranometer in the control is the one receiving the higher proportion of global radiation. Then we have the agroforestry pyranometer on the inter-row in block 1 followed by the two pyranometer on the row (block 2 then block 1) and finally the pyranometer on the

inter-row in block 2 receives the less radiation. This result is unexpected as the inter-row should receive more direct radiation than the row but it is also necessary to consider the relative position of the trees south of the inter-row. In our case the pyranometer has two well-developed walnut trees that are putting it in the shade for most of the day.

We also looked at the daily variation of temperature and relative humidity to see if the agroforestry system acted as a buffer (appendix II). The presence of walnut trees did not act as buffer on relative humidity but had an influence on the temperatures (Figure 12). The daily variation between the minimum and maximum temperature was usually less important in the agroforestry system compared to the agricultural control except for the sensor on the inter-row in block 1. We assume this is due to the sensor being placed at the beginning of the fifth row and that there is a large open space north of it with no trees placing it in the same conditions as the control. The temperature variation in the inter-row in block 2 is equivalent to the variation on the row in block 1 and the variation on the row in block 2 is significantly lower than everywhere else. Looking at these results, the buffer effect on temperature seems to be more important in block 2 than in block 1 probably because of the proximity to the river and a denser vegetation.

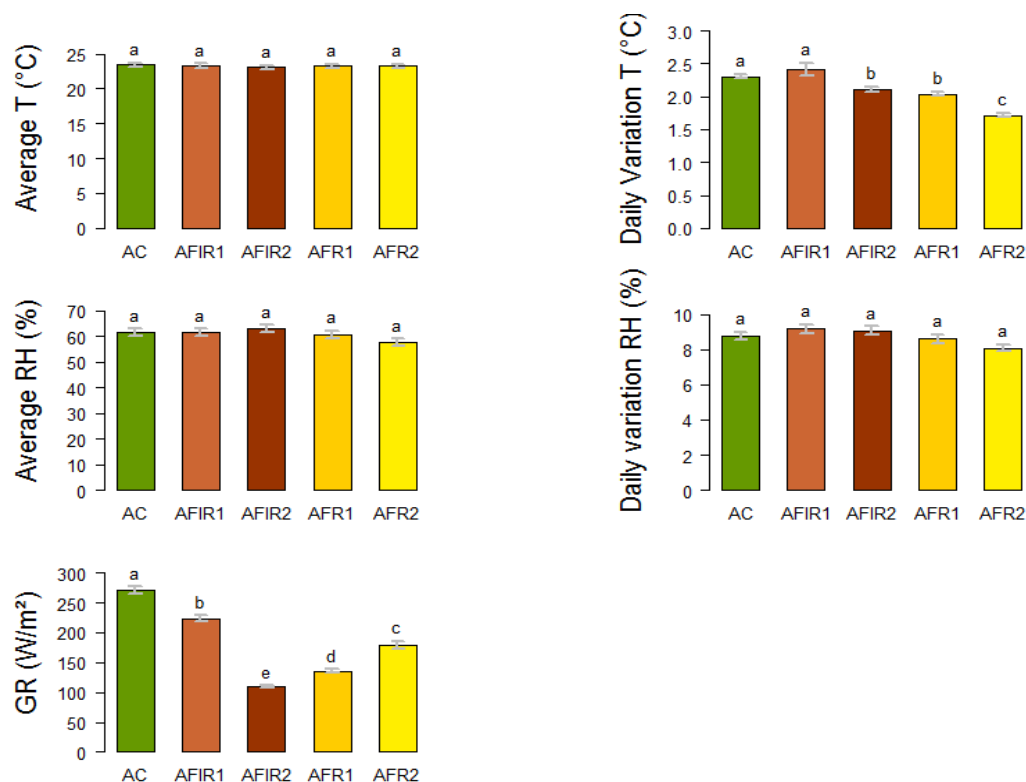


Figure 12: Kruskal-Wallis rank test results on the data collected by the sensors and pyranometers from the A1 plot of domaine de Restinclières.

### III.1.2 The Gap Fraction analysis

The results of the processing of the photographs using a fisheye showed a significant difference for each modality and at the two dates (Figure 13). Globally the two models returned the same value but there was also a small difference. The UOC model returned a lower value for high values (close to one) than the SOC model. That difference was inverted for low values (under 0.5).

The differences between our modalities before the walnut trees budburst models (April) using uniform overcast sky (UOC) and standard overcast sky (SOC), varied from 0.98 for the agricultural control (AC) to 0.83 for the row modality (AF\_R). The inter-row modality was in the middle at 0.93. While significant these differences were still relatively small. At full walnut tree's foliation, at the beginning of June, the differences were exacerbated ranging from 0.97 for the AC to 0.36 for the AF\_R modality and 0.73 for the AF\_IR modality.

#### Synthesis

As supposed the aboveground environment is modified by the presence of the walnut trees. Even if there are no significant differences on absolute values we did show that the trees acted as a buffer on daily temperature variations. We also showed that there was a significant differences in the direct light environment and the global radiation received by the apple trees.

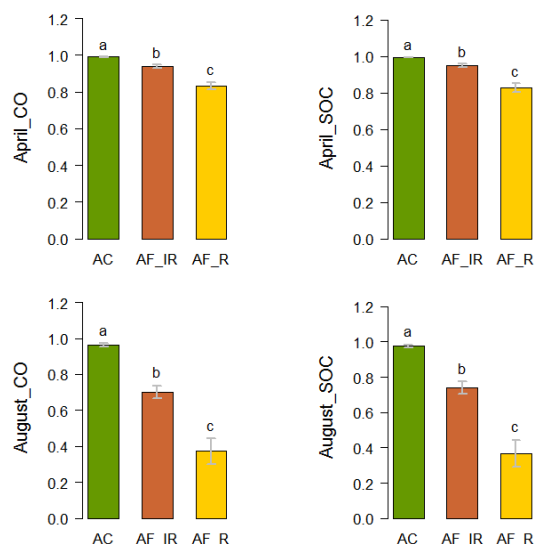


Figure 13: Gap fraction results on two different models used to estimate diffuse light using photographs taken with a fisheye lens. CO stands for uniform overcast sky and SOC standard overcast sky. The pictures were taken from experimental site of “domaine de Restinclières” from the ground next to the apple trees (Dalinette cultivar on G202 rootstocks) before walnut trees bud burst (April) and at full foliation (August). Approximately ten pictures in each modality were analysed (AF\_IR: Agroforestry inter-row; AF\_R: Agroforestry row; AC: Agricultural control). Significant differences between modalities were tested by a Kruskal-Wallis test ( $\alpha = 0.01$ ).

## III.2 Aerial growth and ramification

### III.2.1 Agroforestry limiting water potential?

Looking at pre-dawn water potential (Figure 14) the AF\_R modality is the one presenting the more negative potential (-1.05 MPa) which means that it is where the water is the hardest to absorb. The agricultural control modality is not significantly different from the other two modalities (potential at -0.76 MPa). Finally, the AF\_IR modality is significantly different from the AF\_R modality (-0.57 MPa).

The midday water potential, which represents the plant hydric status when the stress is theoretically the highest, is really different from the one at pre-dawn. There is no longer any significant differences between the modalities. The hydric potential varies from -2.04 MPa for AC to -2.4 MPa in AF\_IR. The highest difference between pre-dawn water potential and midday water potential was observed for the AF\_IR modality (1.83 MPa) while the lowest

difference was for the AF\_R modality with 1.19 MPa and a 1.28 MPa difference for the agricultural control.

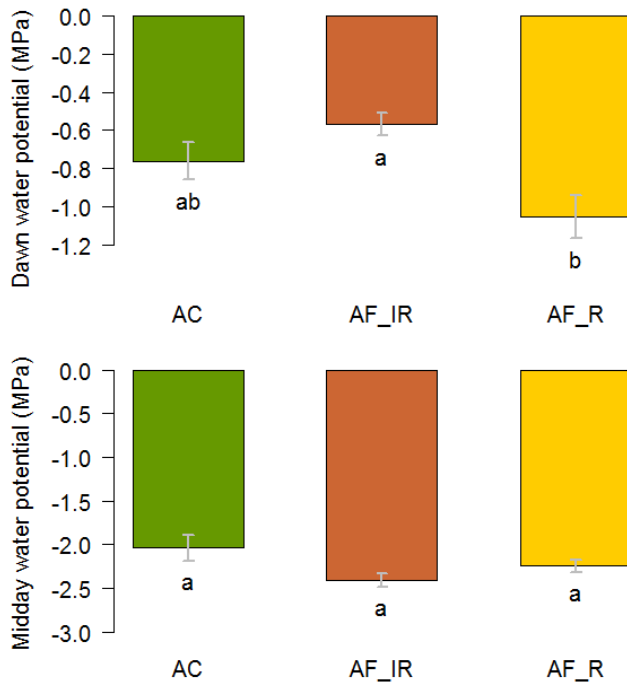


Figure 14: Water potential at pre-dawn and at midday of young apple trees (Dalinette cultivar on G202 rootstocks) in a mature agroforestry system (walnut trees) from the experimental site of “domaine de Restinclières” among the different modality (AF\_IR: Agroforestry inter-row; AF\_R: Agroforestry row; AC: Agricultural control). Fifteen fully grown leaves were measured for each modality. Significant differences between modalities were tested by a Kruskal-Wallis test ( $\alpha = 0.01$ ).

### III.2.2 One year old shoots

#### III.2.2.1 Analysing trees geometry: slenderness and taper

Before looking at the growth during this second year, we measured the diameter and length of the apple-tree stems grown during the first year (2016) to see if there was a significant difference between our modalities. We then analysed slenderness by placing each tree in a two dimensional graph depending on these values (Figure 15).

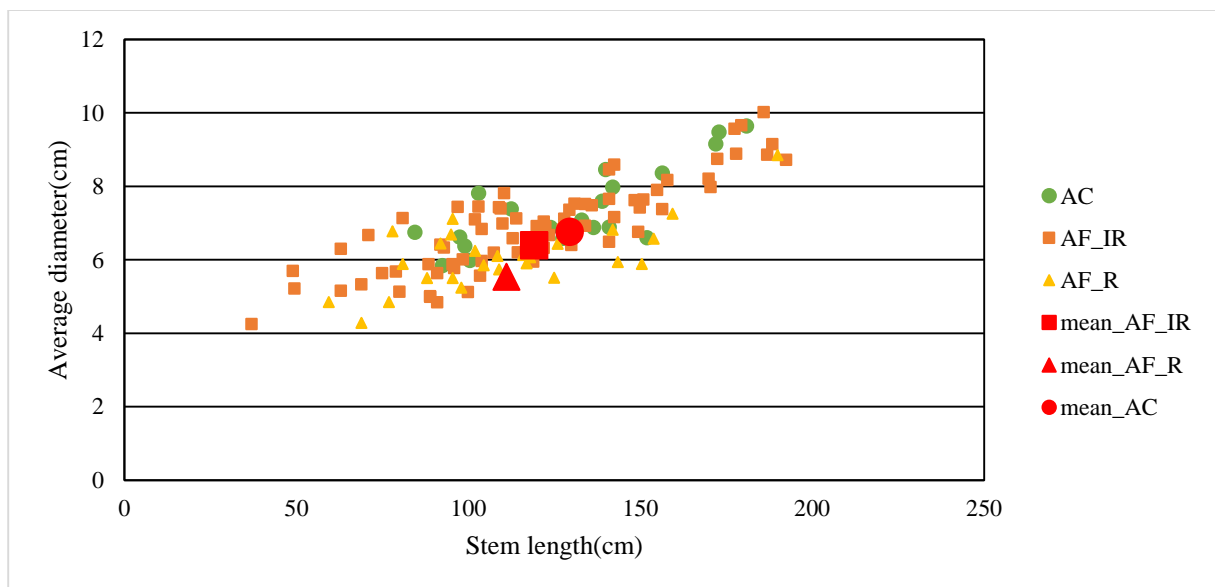


Figure 15: Average stem diameter in relation to stem length after one year growth of apple trees (Dalinette cultivar on G202 rootstocks) in a mature agroforestry system (walnut trees) from the experimental site of “domaine de Restinclières” among the different modality (AF\_IR: Agroforestry inter-row; AF\_R: Agroforestry row; AC: Agricultural control). We also represented an average tree using the average of each modality. Each symbol represents a tree.

At first sight, it looks like that there is no difference between our modalities, even if AF\_R trees seems overall smaller than AC ones. AF\_IR trees present the most heterogeneity with small trees as well as tall ones. We can also note that there is a positive correlation between the diameter and the length of the stem.

The standardised major axis (SMA) regression between average stem diameter and stem length returned the results presented in Table 6.

Table 6: Standardised major axis (SMA) regression between average stem diameter and stem length after one year growth of apple trees (Dalinette cultivar on G202 rootstocks) in a mature agroforestry system (walnut trees) from the experimental site of “domaine de Restinclières” among the different modalities (AF\_IR: Agroforestry inter-row; AF\_R: Agroforestry row; AC: Agricultural control). By convention, relationships are in the form Y vs. X. All analyses are on non-transformed data except for  $P_{slope=1}$  where there are log10 transformed. In **modalities characteristics**, n: sample size; slope: slope of the relationship;  $r^2$ : coefficient of determination of the SMA regression;  $P_{slope=1}$ : probability associated to isometries (null hypothesis  $H_0=1$ ). In **test results**, we present each test that was made on our data and their associated p-value. Outputs of the SMA regression can be seen in appendix III.

### Modalities characteristics

Modality	n	slope	$r^2$	$P_{slope=1}$	Type of allometry
AC	21	0.044	0.612	0.151	Isometry ( $\log_{10}$ slope = 0.80)
AF_IR	74	0.035	0.652	<0.01	- ( $\log_{10}$ slope = 0.59)
AF_R	26	0.031	0.372	<0.01	- ( $\log_{10}$ slope = 0.62)

### Test results

Test for common slope	>0.01
Test for shifts in elevation	>0.01
Test for shifts along the common slope	<0.01

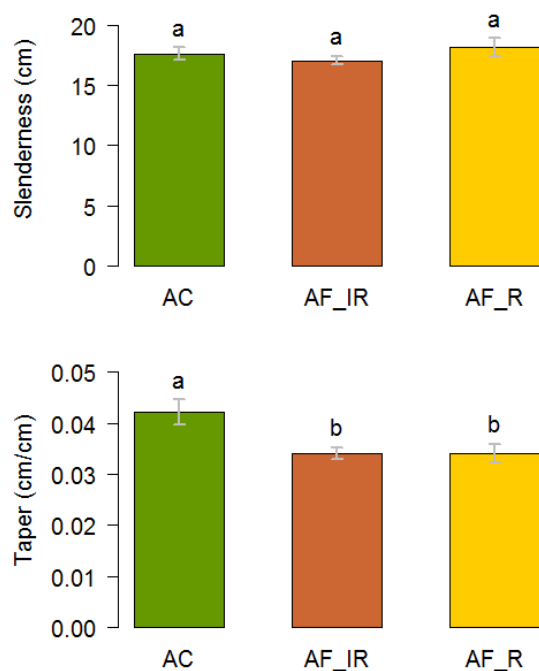


Figure 16: Analyse of two indicators used in forestry, slenderness (cm of stem / cm in diameter) and taper (cm loss of diameter / cm of stem), of young apple trees (Dalinette cultivar on G202 rootstocks) in a mature agroforestry system (walnut trees) from the experimental site of “domaine de Restinclières” among the different modalities (AF\_IR: Agroforestry inter-row; AF\_R: Agroforestry row; AC: Agricultural control). Significant differences between modalities were tested by a

For the agricultural control there was an isometry as the null hypothesis was that the slope was equal to one. But there was a negative allometry for the apple trees in the agroforestry system, the stem diameter evolves disproportionately compared to the stem length. In other words, the stem length increased faster than the stem diameter.

Only the test for shifts along the common slope gave a significant difference between AF\_R and AC (p-value < 0.01) indicating higher length and diameter values for AC stems. There was no significant difference between AF\_IR and AF\_R or AF\_IR and AC.

We used another approach of slenderness considering an average value of all stems whereas the SMA approach describe the bi-variables variations. For the slenderness (Figure 16), surprisingly considering we have shown that there was an allometry, there is no significative difference between our modalities.

Looking at the taper, there is a significative difference between our modalities. The apple trees in our agricultural control lose 0.04 centimetres per centimetre of stem as the apple trees in the agroforestry system (on the row and inter-row) are only losing roughly 0.03 centimetre per centimetre of stem.

In other words, after one year of growth, the apple trees in the agricultural control are more conical than the apple trees in the agroforestry system.

### III.2.2.2 Analysing architecture: buds type and topology

As we were following the apple trees growth rates we also looked at their topology, at their bud's type and organisation.

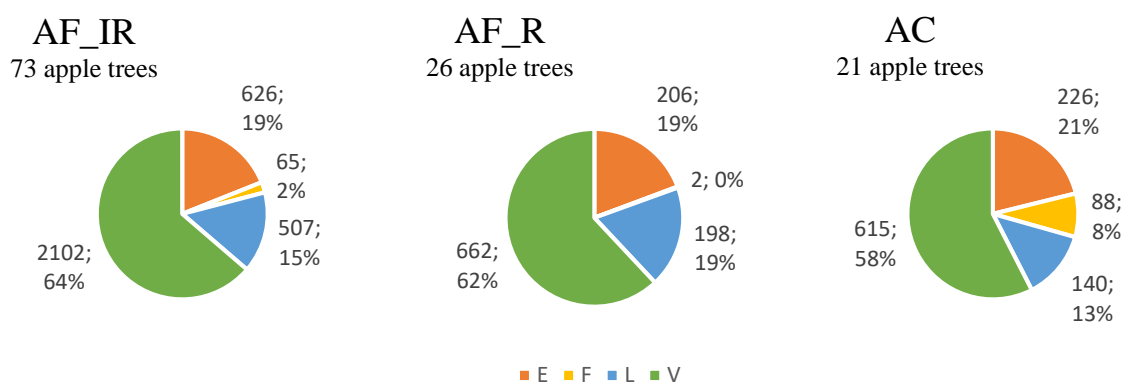


Figure 17: Bud type distribution (E = Extinct; F = Flower; L = Latent; V = Vegetative) of young apple trees (Dalinette cultivar on G202 rootstocks) in a mature agroforestry system (walnut trees) from the experimental site of “domaine de Restinclières” among the different modality (AF\_IR: Agroforestry inter-row; AF\_R: Agroforestry row; AC: Agricultural control)

Out of the 149 apple trees only 120 (73 apple trees in the AF\_IR (Agroforestry inter-row) modality, 26 in the AF\_R (Agroforestry rows) and 21 in the AC (Agricultural control) that were evenly distributed between the 2 blocks) were analysed: the others were eliminated because of accidents while manipulating them or mortality (Figure 17). Percentage of vegetative bud are more important in apple trees that are on the inter-row of walnut trees (64%) compared to the apple trees that are on the row of walnut trees (62%). More importantly, apple trees in the agroforestry system, IR and R, have both a higher vegetative bud rate that the apple trees in the agricultural control (58%). This observation could be explained by the fact that there are more flower bud in the control (8%) compared to the apple trees in the agroforestry system (2% and 0.2%).

Apple trees that are in the most competitive environment (AF\_R) are also the trees that have the most latent buds, 19% against 15% for the apple trees in between the rows of walnut trees and 13% for the control. Finally, the ratio of buds that are extinct is similar in the agroforestry system (19%) and less important than in the control (21%).

Overall, the differences between the modalities seemed to be rather small. Using a Kruskal-Wallis test, we looked if there were any significant differences, 8 weeks after bud burst, on the buds type between the apple trees in the different modalities (Figure 18).

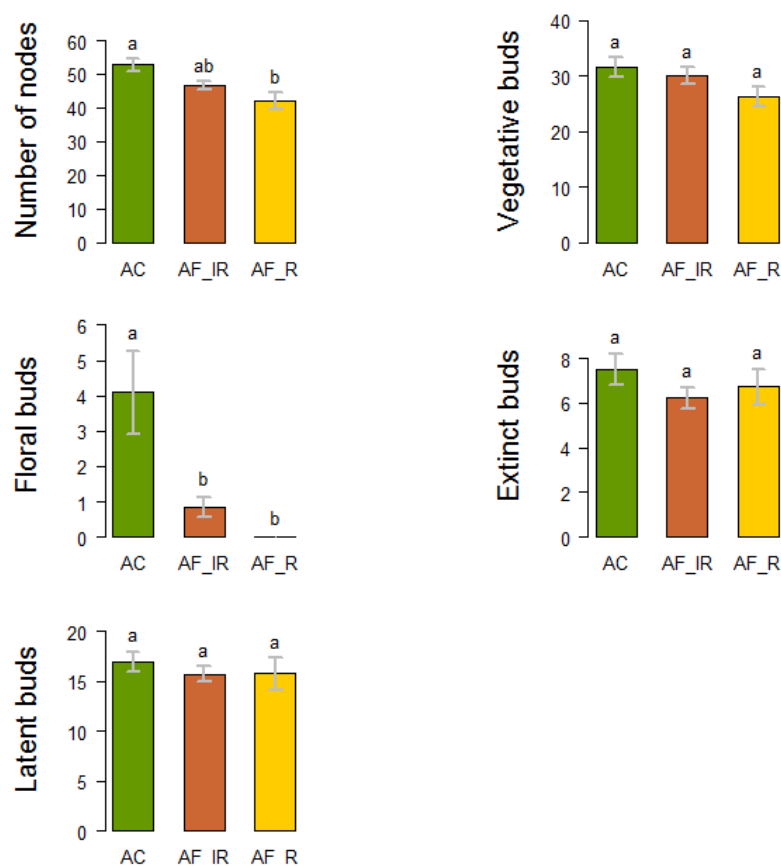


Figure 18: Analyse results on the number of nodes and the different type of buds of young apple trees (Dalinette cultivar on G202 rootstocks) in a mature agroforestry system (walnut trees) from the experimental site of “domaine de Restinclières” among the different modalities (AF\_IR: Agroforestry inter-row; AF\_R: Agroforestry row; AC: Agricultural control). We represented the standard deviation and the statistical group for each modality. Significant differences between modalities were tested by a Kruskal-Wallis test ( $\alpha = 0.01$ ).

There was no difference looking at the latent, vegetative or extinct buds. But there were significantly more nodes on an apple tree in the agricultural control (AC), with an average of 52 nodes, compared to the apple trees in the agroforestry system and on the rows of walnut trees (AF\_R), average of 42 nodes. The apple trees between the rows of walnut trees (AF\_IR), average of 48 nodes, in the agroforestry system are not significantly different from the two other modalities. Looking at flower buds, there are significantly more profuse in the agricultural control (AC), average of 4 flowers per tree, compared to the other two modalities, with less than one flower per tree for AF\_IR and none for AF\_R.

We didn't see any differences between our modalities looking at vegetative and latent buds topological distribution along the parent stem. Latent buds are mainly located near the base of the trunk while vegetative buds were located everywhere but the base. Looking at extinct buds, it does not like there is a difference in the number of extinct buds between the apple trees growing in the agroforestry system and the one in the agricultural control bud a difference in their relative position on the stem. The extinct buds in the agroforestry system are located everywhere equally but the first 20% of buds at the apex while in the agricultural

control most of the extinct buds are located in the bottom half of the apple tree. Finally, for the flower buds, as they were less than 5 flower buds on apple trees on the row in the agroforestry (AF\_R) system we did not consider them in our analysis. Between the apple trees on the inter-row (AF\_IR) and the agricultural control (AC) the relative position of the flower buds is similar except that there was more flower terminal buds in the agroforestry system than in the agricultural control (Figure 19).

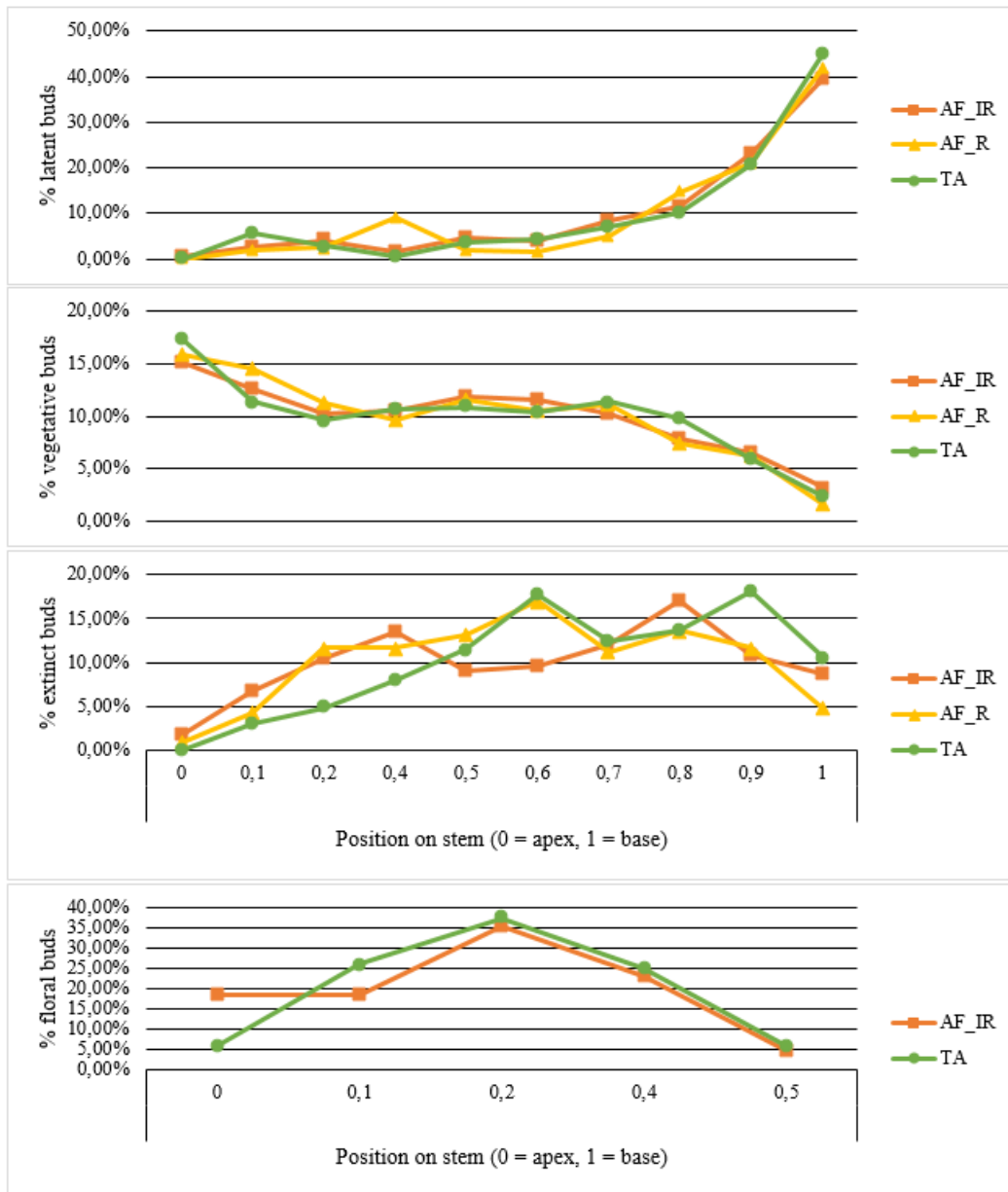


Figure 19: Topology of the different type of buds on the stem of apple trees in their second year growth (Dalinette cultivar on G202 rootstock). 0 represents the apex of the stem while 1 represents the base. We represented the relative position of buds and made 10 classes regrouping all buds by steps of 0.1. We only considered a modality when they were more than 5 buds of the same type. The apple trees are from the experimental site of “domaine de Restinclières” in which we studied three different modalities (AF\_IR: Agroforestry inter-row; AF\_R: Agroforestry row; AC: Agricultural control).



### III.2.2.3 Analysing phenology: flower buds

Having no flower buds in AF\_R modality, we could only look at the difference between AC and AF\_IR modalities (Table 7).

Table 7: Ordered logit regression t and p values of flower bud phenology for each week after bud burst. Measurements were made on young apple trees (Dalinette cultivar on G202 rootstocks) in a mature agroforestry system (walnut trees) from the experimental site of “domaine de Restinclières”. Agroforestry inter-rows (AF\_IR) modality is compared to a reference, agricultural control (AC). Outputs of the ordered logit regression regression can be seen in appendix IV.

	Week1	Week2	Week3	Week4	Week5	Week6	Week7	Week8
<b>t-value</b>	-0.17	-0.06	0.14	-0.15	0.002	-	-	-
<b>p-value</b>	0.58	0.84	0.65	0.69	0.99	-	-	-

A negative t-value means that the flowers in the AF\_IR modality are less advanced compared to the agricultural control. Another way to say this is that there is more advanced phenological stage in the AC modality compared to the AF\_IR. However, the p-value indicates that there are no significant differences for every date between the two modalities from bud burst to fruit set.

## III.2.3 2017's growth results

### III.2.3.1 Apple trees growth dynamics

Only trees that kept growing were taken into account when looking at the apple trees growth therefore our study consisted first in analysing the proportion of growth arrest through the season, and second to analyse for the growing shoots their increase in the number of nodes and in length. During the first ten weeks, there were no differences in the percentage of growth arrest between the apple trees in our different modalities and the percentage stayed under 20% (Figure 20). After this date, there was clearly a difference between AC and AF (IR and R). While the apple trees in the control did not suffer any increase in growth arrest there was over 20% of apple trees that had a growth arrest in the agroforestry system (IR and R). The proportion of growth arrest was stable for 4 weeks in the different modalities before sky rocketing in two weeks to approximately 70% for the agricultural control and 80% for the agroforestry system. The last week, the proportion of growth arrest decreased for the control and the apple trees on the inter-row and kept increasing for the apple trees on the row.

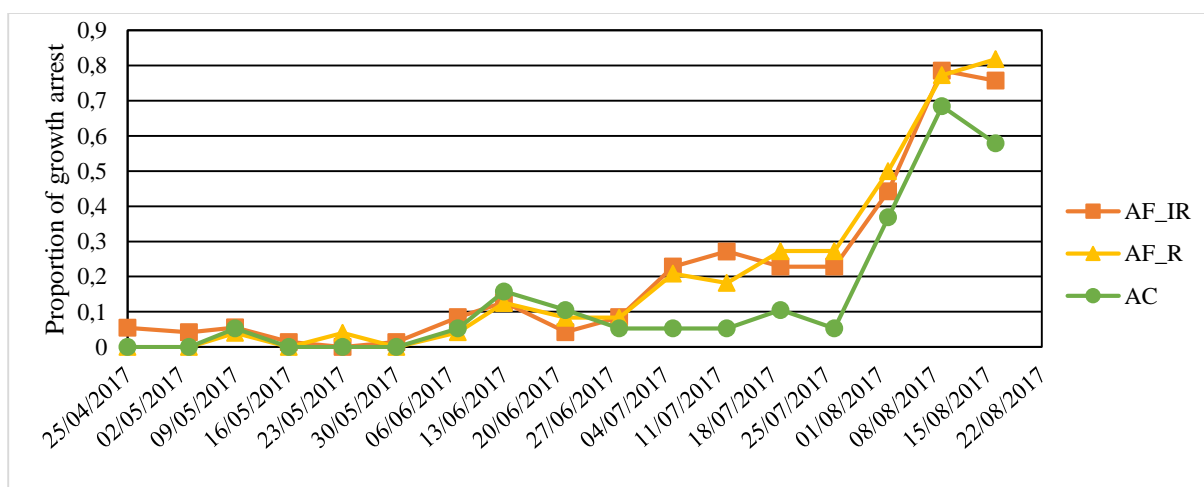


Figure 20: Proportion of growth arrest of two-year-old apple trees (Dalinette cultivar on G202 rootstocks) in a mature agroforestry system (walnut trees) from the experimental site of “domaine de Restinclières” among the different modalities (AF\_IR: Agroforestry inter-row; AF\_R: Agroforestry row; AC: Agricultural control).

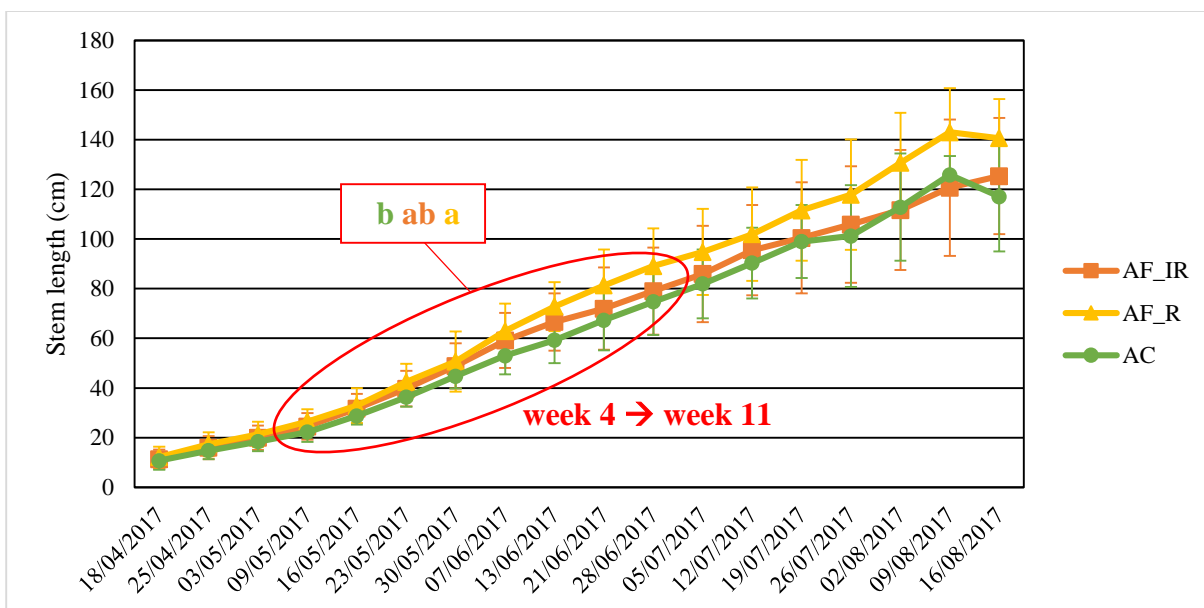


Figure 21: Shoot length grown the second year (mean±SD) of two-year-old apple trees (Dalinette cultivar on G202 rootstocks) in a mature agroforestry system (walnut trees) from the experimental site of “domaine de Restinclières”. The stem length was measured each week for 18 weeks. We used an ANOVA when possible or a Kruskal-Wallis rank test ( $\alpha = 0.01$ ) to highlight significant differences between our three modalities: AF\_IR: Agroforestry inter-row; AF\_R: Agroforestry row; AC: Agricultural control. Statistical analysis are shown in appendix V.III.

There was not significant differences in the stem length increase among the three modalities before the fourth week and this difference maintained until the eleventh week (Figure 21). The shoot of the apple trees on the row was significantly longer than the shoot of the apple trees in the agricultural control. The apple trees on the inter-row were not significantly different from either modality.

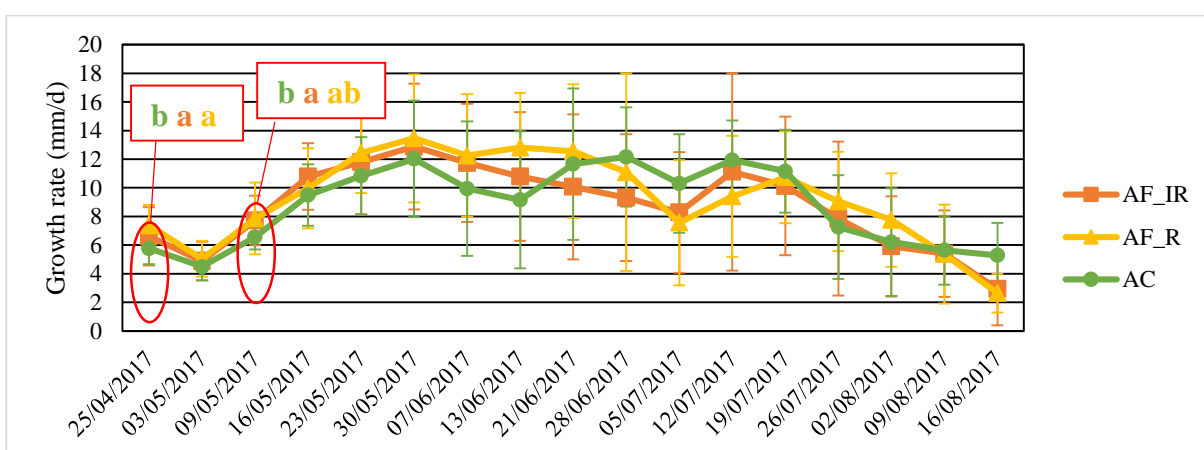


Figure 22: Growth rate of the shoot (mean±SD) of two-year-old apple trees (Dalinette cultivar on G202 rootstocks) in a mature agroforestry system (walnut trees) from the experimental site of “domaine de Restinclières”. We used an ANOVA when possible or a Kruskal-Wallis rank test to highlight significant differences between our three modalities: AF\_IR: Agroforestry inter-row; AF\_R: Agroforestry row; AC: Agricultural control. Statistical analysis are shown in appendix V.III.

After the eleventh week there is no longer any differences between our modalities even if it looks like the gap between the curves seems to increase. However this is not due to the standard deviation increasing as there was no significant changes in the coefficient of variation from week eleven onwards (appendix V.I).

There was no significant differences in the number of leaves throughout the weeks suggesting that the apple trees have the same plastochrone independently of the modality (appendix V.II).

The apple trees in the agroforestry system had a growth rate significantly higher than the apple trees in the agricultural control between the first and second week (Figure 22). Between the third and fourth week, only the apple trees in the inter-row in the agroforestry were significantly different from the apple trees in the control even though the growth rate was the highest for the apple trees on the row. But the statistical analysis did not show any significant differences probably because of a standard error being too high for the AF\_R modality.

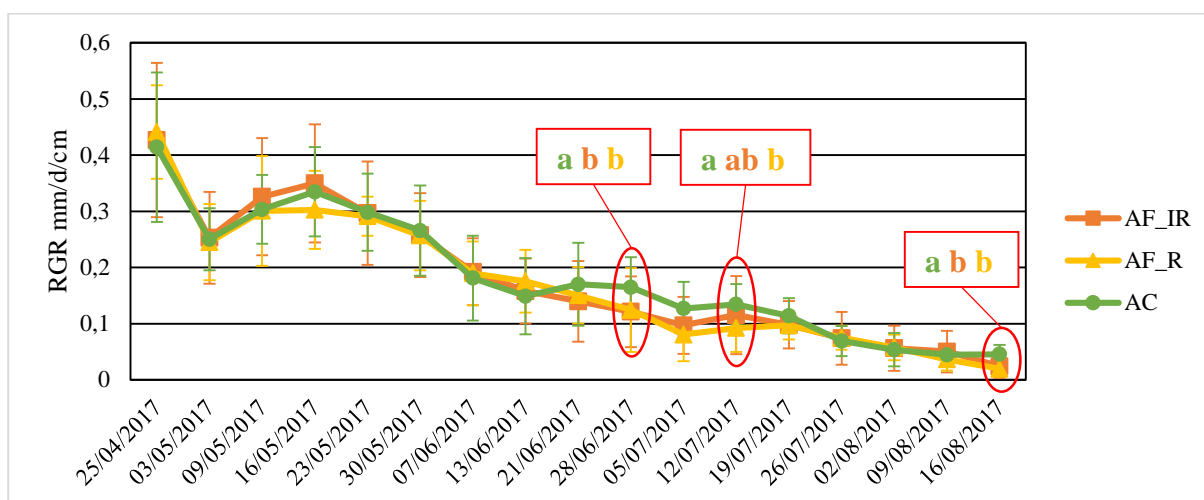


Figure 23: Relative growth rate of the shoot (mean±SD) of two-year-old apple trees (Dalinette cultivar on G202 rootstocks) in a mature agroforestry system (walnut trees) from the experimental site of “domaine de Restinclières”. We used an ANOVA when possible or a Kruskal-Wallis rank test to highlight significant differences between our three modalities: AF\_IR: Agroforestry inter-row; AF\_R: Agroforestry row; AC: Agricultural control. Statistical analysis are shown in appendix V.III.

Looking at the relative growth rate there were significant differences between the weeks 10-11, 12-13 and 17-18 (Figure 23). The relative growth rate of the apple trees in AC was significantly higher than in the other two modalities. That difference was mainly due to the differences in growth rate dynamics as we can see in Figure 22.

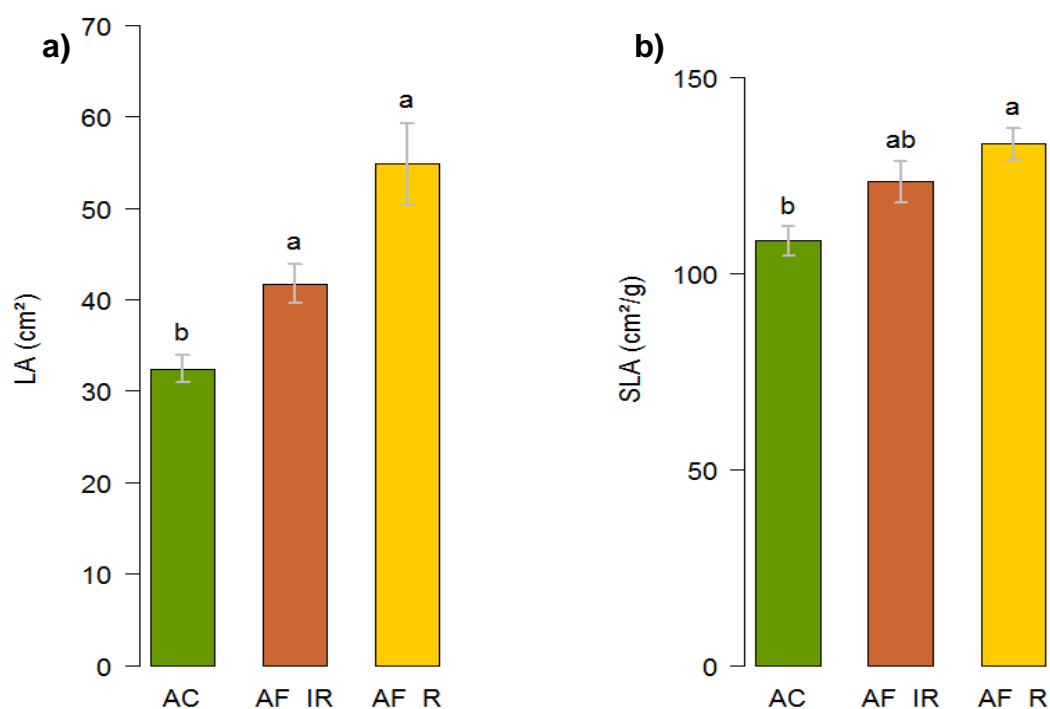
III.2.3.2 *Shade influence on leaves*

Figure 24: Results of leaf area (a) and the specific leaf area (b) analysis of 2-year-old apple trees (Dalinette cultivar on G202 rootstocks) in a mature agroforestry system (walnut trees) from the experimental site of “domaine de Restinclières” among the different modalities (AF\_IR: Agroforestry inter-row; AF\_R: Agroforestry row; AC: Agricultural control). We represented the standard deviation and the statistical group for each modality. Significant differences between modalities were tested by a Kruskal-Wallis test ( $\alpha = 0.01$ ).

Both for LA and SLA there was significant differences between the modalities. LA of AF\_R (54.8 cm²) and AF\_IR (41.7 cm²) was significantly higher than LA of AC (32.4 cm²).

SLA of AF\_R (133 cm² per gram of dry matter) was significantly higher than SLA of AC (108 cm² per gram of dry matter) with SLA of AF\_IR in intermediate position (123 cm² per gram of dry matter).

### *Synthesis*

As expected, the presence of the walnut trees influences the growth of the apple trees. After one year the differences between the modalities were slim even if it seemed like the AF\_R trees were smaller. At first it looked like there was etiolation in the agroforestry system that was not confirmed by the slenderness analysis made on an average value of all stems. However, the SMA analysis indicates a negative allometry relation between diameter and stem length, the stem increased faster than the diameter. However there was a difference in taper meaning that the trees average diameter in the control was higher than in the agroforestry. There was also a difference in the total number of nodes and the number of flower buds. During this growing season, the differences in stem length and growth rate were more marked in the early season but disappeared quickly with the increase of growth arrest in the agroforestry system. There was also a difference in leaves morphology, with a significantly higher leaf area and specific leaf area in the agroforestry system compared to the control. Agroforestry also modifies the daily evolution of water potential, a shady environment limits the midday hydric stress.

## IV. Discussion

### IV.1 Consequences of a modified environment on the apple tree architecture and growth

The presence of walnut trees modifies the aboveground environment of the apple trees: (i) buffers daily temperature variations, (ii) limits incoming light and global radiation. While the apple trees were growing at the same speed (identical plastochrone), these modifications induced differences in internodes length during the first weeks (i.e. significant difference in length but not nodes) and proportion of growth arrest one month after walnut trees bud break.

The microclimate close to the walnut trees induced more negative pre-dawn water potential without affecting midday leaf water potential. Water potential at pre-dawn is equal to the potential of water in the soil as the plant is at parity with it while midday water potential is looked at the water potential when hydric stress is at its maximum (Naor et al. 1995; Naor et al. 2008). Our results confirmed this trend, as an average over our three modalities water stress is lower at pre-dawn than at midday. Water potential was more negative closer to the walnut trees at pre-dawn indicating that there was a higher water stress close to the walnut trees. The competition for water with the walnut tree is probably increasing when distance decreases therefore increasing the chances of hydric stress for the apple trees if not irrigated. Dehydration have short term and long term effect. On a short term dehydration can be responsible for stomata closure leading to a decrease in photosynthesis assimilates (Chaves et al. 2003). On a long term dehydration can lead to aerial growth arrest and a decrease in transpiration (Chaves et al. 2003). Even if the apples trees are irrigated, it could explain why we observe more growth arrest in the agroforestry system and, therefore, why there was significantly more nodes in the agricultural control the first year where they had irrigation issues. However, the water potential values for leaves obtained after water had been withheld for four days were identical to values from the literature and were not correlated to stress that could lead to growth arrest (Naor 1998). These growth arrest are probably the consequence of the addition of different abiotic stress.

Apple trees are considered isohydric (Lakso 2014) as they have shown a strong stomata response to high vapour pressure deficit (VPD). High VPD is well correlated to high temperature so the presence of walnut trees acting as a buffer on high temperatures will reduce the VPD therefore increasing the time before stomata closure and the quantity of CO<sub>2</sub> for photosynthesis.

In this regard we see three different hypothesis to explain the buffer effect of the agroforestry on water potential between the modalities at pre-dawn and midday. This could be (i) the consequence of a lower VPD, (ii) the fact that the trees closer to walnut tree at pre-dawn are already limiting their transpiration by closing their stomata or (iii) linked to hydraulic movements related to the presence of the walnut tree (hydraulic lift) (Eissenstat & Caldwell 1988; Jose et al. 2004). As these three hypothesis are not antagonist there are possibly all happening simultaneously.

Currents models establish that flower induction is the output of a set of ordered events that originate in the leaf (Bernier 2013). It is now widely accepted that the multifactorial florigen originating in the leaf and triggering the flower transition in the shoot apical meristem or in

the apical meristem includes not only the FLOWERING LOCUS T (FT) protein, but also, among other compounds and depending on the species, plant growth regulators such as gibberellins (GA) or cytokinins of leaf origin (Bernier 2013). Moreover, it is now well known that sugars are involved in flower transition (Yang et al. 2011) and sugar synthesis is highly related to leaf area acting as a source. Apple trees in agroforestry had leaves with a higher specific leaf area (SLA) and so possibly a higher total leaf area because of the lack of light (Lacube et al. 2017). However, flower transition being multifactorial and lacking precise data on 2016 leaf area it is hard to conclude as to why we observed differences on the number of flower buds between our modalities. There were no differences in the phenology of flowers as there was no differences in temperature and so accumulated degree day.

The lack of incoming light and radiation had different morphological consequences. Apple trees in the agroforestry system have and more specifically on the row of walnut trees tend to have smaller trunks (in length and diameter) with a trend towards slender shape. Apple trees in agroforestry tend to invest more energy to primary growth than in secondary growth as if they were looking for light. This is also suggested by the higher extinction rates of buds near the apex in agroforestry apple trees. Extinction of buds can occur to favour other growing point (Lauri et al. 1995) in our case the shoot apical meristem. The apple trees in agroforestry had higher growth rate and therefore longer stem during the first weeks. But, when the walnut trees reached full foliation around the beginning of June, the agroforestry apple trees had a decrease in growth rate (relative growth rate is lower in agroforestry system at the end of June). A physiologically based study showed that shading reduced nutrient uptake (Cadwell 1992); this could also be another factor responsible for this decrease. This was confirmed by the SMA analysis on slenderness (negative allometry) even though there was no differences on slenderness using an average approach. However, if we had used a sample selecting only the tallest trees we might have been able to show a significant difference. The difference in taper still suggests that the apple trees in the agricultural control had a secondary growth more important either because they had a longer growing period and/or a higher photosynthesis yield. These observations might be confirmed with data on the 2017 annual growth.

Some of the observed differences in aerial growth strategy are the consequence of the shade as confirmed by the literature. However, the reality is probably more complex. In fact, the underground interactions between the roots systems have probably played a role in the growth pattern of the apple trees. As we hypothesised, the walnut tree roots could have helped maintain a lower soil water potential at midday by favouring water movement.

## IV.2 Possible future improvement

### *On the methodology*

The design composed of three modalities was not satisfying. The important heterogeneity within each modality can possibly have concealed differences. That could explain why we were not able to show an effect of the block even though the environmental conditions were significantly different. Therefore we propose that the apple trees should not be grouped into modalities but rather that each tree is characterised independently using an indicator of competition (Fichtner et al. 2017) that could be composed of: (i) the distance to the walnut trees that can possibly have an aerial interaction with the tree and their geographic position to the tree, (ii) trunk diameter that can be correlated to the size of the trees crown (Hemery et al.

2005) and root spread (Gilman 1989), (iii) the gap fraction of each tree with a simulation of the course of the sun over the year. To go further it would be interesting to try different thinning methodology on the walnut tree to try and limit negative impacts on the apple trees (i.e. pollarding versus natural shape).

The HMP sensors and pyranometer were installed late on the plot therefore the data collected were only on a short time period. It would be interesting to leave the sensors permanently on the plot to generate more data over the whole growing season. The lack of time and knowledge in analysing meteorological data was also a problem. By making a daily average of the collected data we did not take into account the day/night alternation for temperatures and radiations. Furthermore when we compared the data with a Kruskal-Wallis test rank we did not compare day to day data but rather a sample over a course of time to another sample possibly losing in the process some information. It would then have been possible to correlate the meteorological data to growth dynamics.

The 2017's growth data statistical analyse was only conducted on trees that did not suffer of growth arrest (i.e. stem growth superior to 0.5 cm) therefore possibly reducing the sample size each weeks. Even though the proportion of growth arrest was under 30% for the majority of the experiment the last two weeks the proportion were above 70%. It is then possible that our results lacks representativeness and that our statistical analysis lacks power increasing the probability of a false negative.

#### *On the field management*

The thinning of the ramifications below the first wire could have had an impact on the growth of the stem. The apple trees in the agroforestry were globally smaller than the ones in the control with more branches at the bottom of the trunks. We did not collect the data but it looks like there is longer shoots on the apple trees in the agricultural control compared to those in the agroforestry. It is possible that by thinning the lower shoots we eliminated most of the long shoots of some apple trees thereby favouring the growth of the shoot by decreasing the internal competition for assimilates. In that case, the difference in growth rate and shoot length highlighted during the first weeks could be a consequence of this intervention rather than an effect of the environment. However, even if we chose to let the trees take a natural shape there are still some intervention that needs to take place like tree training by attaching them to the wires.

Even if we tried to reduce all limiting factors induced by the belowground interactions not taking them into account and considering them negligible was a strong hypothesis even though there is no prove that there is an additive effect of above and belowground competition (Casper & Jackson 1997). In contrast to aboveground competition which primarily involves a single resource, light, plants compete for a broad range of soil resources, including water and at least 20 essential mineral nutrients that differ in molecular size, valence, oxidation state, and mobility within the soil. Belowground competition often reduces plant performance more than does aboveground competition (Wilson & Tilman 1991). While it is true that belowground competition often decreases with increases in nutrient levels (Casper & Jackson 1997), we are not sure that we provided enough nitrogen to ensure that we reduced competition under levels that would not affect the apple trees growth. Therefore it would have been interesting to have measurements of belowground competition by combining exclusion tubes and resource based approaches (Casper & Jackson 1997). However, as it is not possible

to use exclusion tubes once the apple trees are planted it also possible to estimate the competition using the root traits (Casper & Jackson 1997; Bardgett et al. 2014).

Juglone (5-hydroxy-1,4-naphthoquinone), the phenolic compound responsible for black walnut allelopathy, has not been taken into account. While it is true that no study have been made on the rootstock (G202) behaviour and tolerance to juglone, black walnut allelopathy has been documented to affect growth of various vegetables, field crops, ornamental plants and several woody species (Jose & Gillespie 1998a). Furthermore it has been shown that a significant amount of juglone can affect seedlings and growth build up in the soil within a ten year period in an alley cropping system (Jose & Gillespie 1998b). However the quantity of juglone recovered in the soil decreased drastically two meters away from the trees (Jose & Gillespie 1998b) and it is difficult to compare chloroform extractable juglone levels to those actually experienced by plants in the field. Yet, in our alley cropping system being over twenty-years-old we can assume that the quantity of juglone accumulated in the soil are higher than those reported in this study. It could be relevant to measure the quantity of juglone accumulated spatially in our experiment and measure the negative effect on the apple trees growth *in vivo* or at least take into account the possible negative effect of juglone on the growth of the apple trees while interpreting our results.



## Conclusion

This study showed that the apple trees adopted different growth strategy in relation to the modification in their aboveground direct environment (Figure 25).

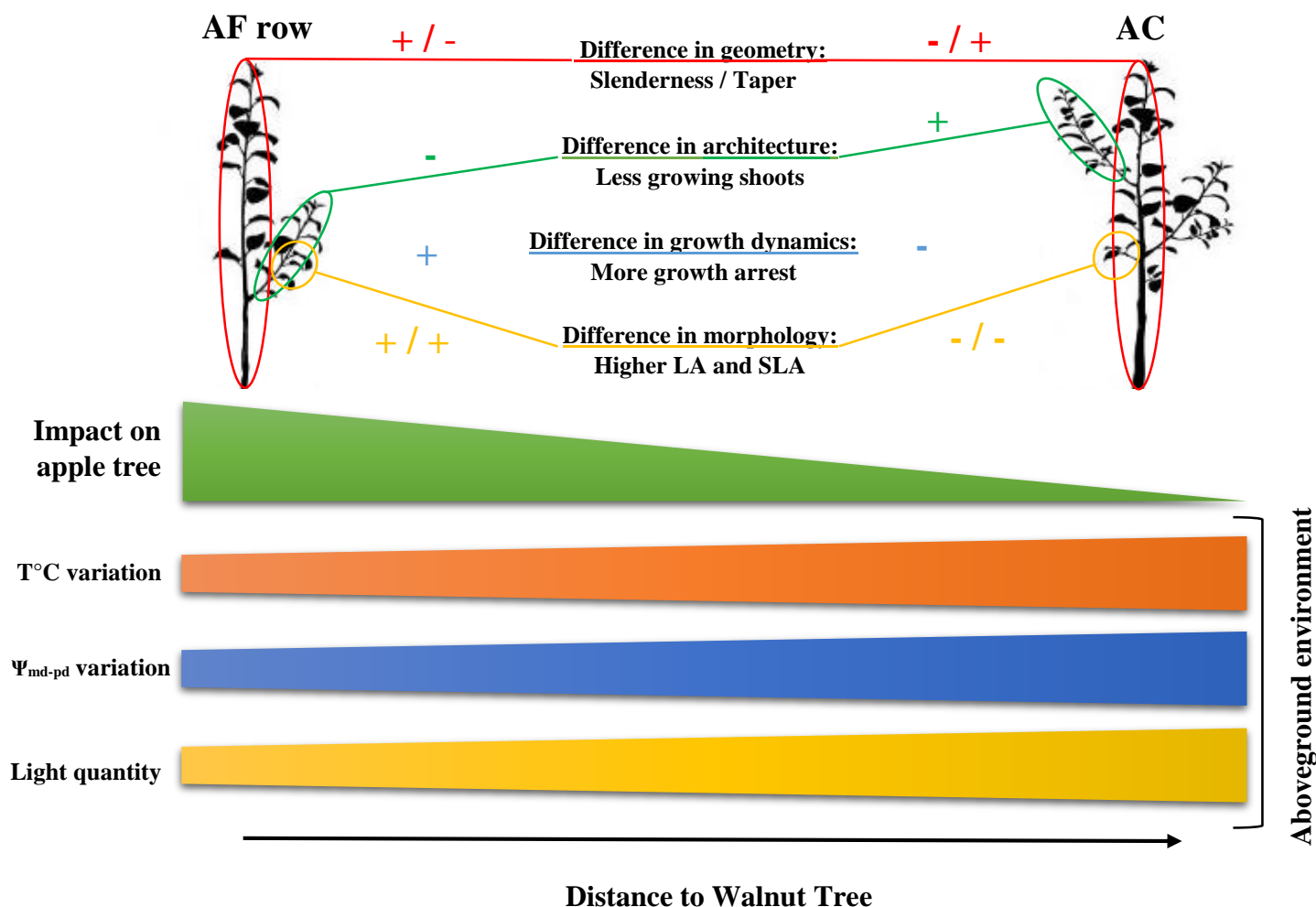


Figure 25: Scheme summarising the growth strategy of the apple tree in relation to its distance to the walnut tree based on the results of this study.

However it is important to keep in mind that the end goal of the fruit-tree is to bear enough fruit in quantity and quality to generate an income. Fruit set and early fruit development (leading to final fruit size and yield) rely on supply of carbohydrates and nitrogenous compounds which are sourced from reserves and current photosynthesis. During the first weeks after bloom, carbohydrate demand from developing shoot and fruit sinks is likely to be greater than supply, and shoot development is thought to have priority for limited carbohydrate supply over fruit development. This competition among sinks is believed to result in fruit abscission (Lakso et al. 1999; Lakso et al. 2006). Therefore, it will be necessary to measure the impact of the observed differences on fruit setting and fruit abscission to see if the trees growing in the agroforestry system have the potential to reach satisfying yields.

During the thesis that will follow this work, xylem sap fluxes will be measured and correlated to the development (architecture, bloom and fruit set) of the apple trees therefore opening a path to understanding how they adapt to their environment.

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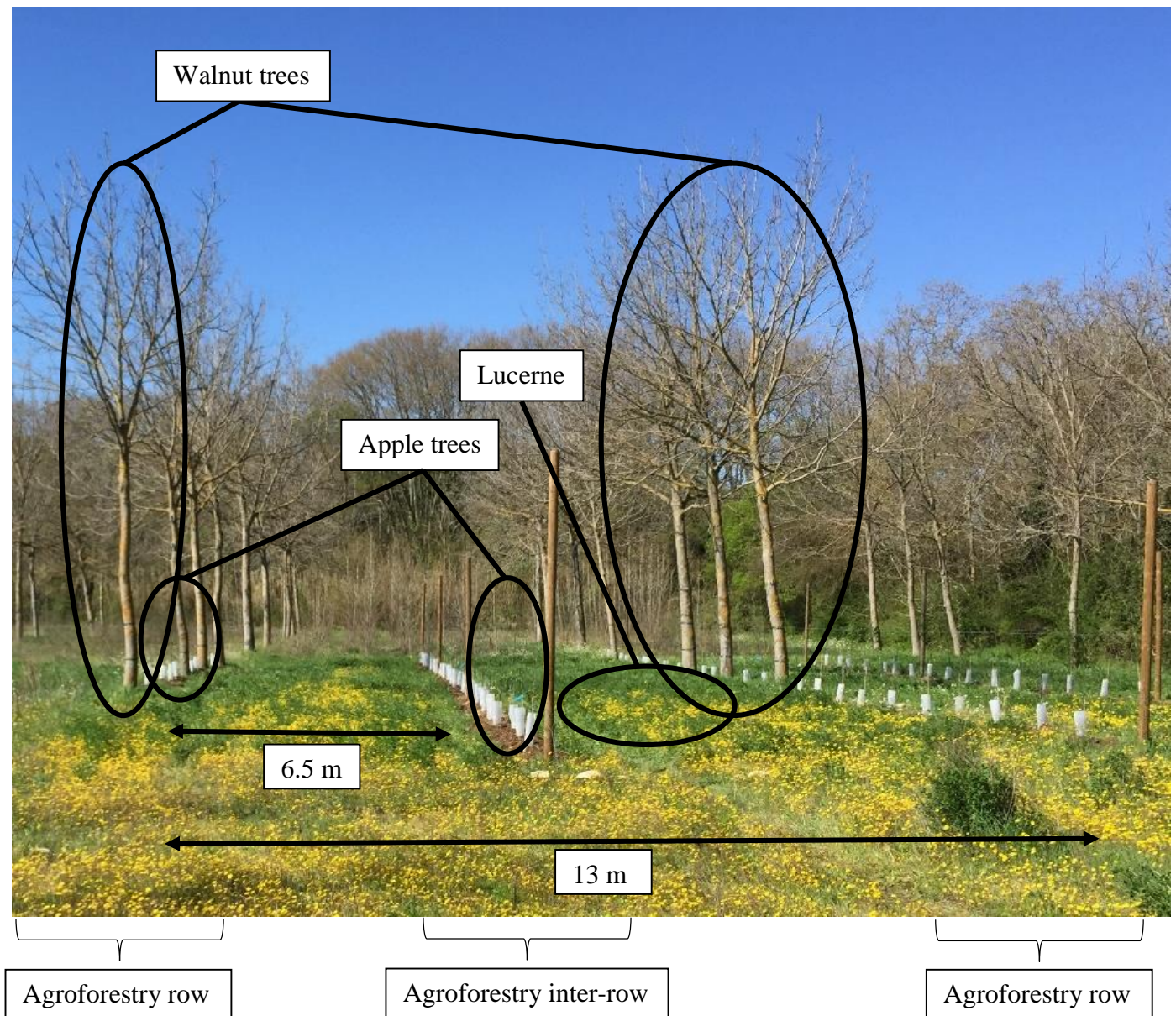
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## Appendix

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### Appendix 0: Apple-Agroforestry Experiment



## Appendix I: *In situ* rhizotron methodology

To follow apple trees root growth we installed three rhizotron (one per modality). We chose three trees that were well developed.

We dug three holes using a compact excavator to 160 centimetres. We then inserted a 1m\*1m polymethyl-methacrylate (PMMA) glass that was hold in place by a metal frame on the side of the apple tree (Photograph 4). The gap behind the glass was filled with sieve earth that had been excavated.

The acquisition of root images were made every week using a scanning application on smartphone (CamScanner INTSIG Information Co., Ltd, Shanghai, China, version 3.9.5) as it has been shown that it has good image accuracy and is faster and cheaper to use than a flatbed scanner (Mohamed et al. 2017). The CamScanner application automatically detects object borders and removes background noise using image-processing technologies. This software adjusts image details, brightness and contrast and can return processed data in a JPG or PDF format. The smartphone was always placed at the same place before taking the picture and a fixed scale (measuring tape) was scanned simultaneously to calibrate the scan.



Photograph 4: *In situ* rhizotron in the apple-agroforestry experiment on plot A1 of the “domaine de Restinclière” (picture by Benjamin Pitchers).

Once images of root growth had been acquired, we conducted analyses of images using the semi-automated SmartRoot software. SmartRoot is an operating system independent freeware based on ImageJ and uses cross-platform standards (RSML, SQL, and Java) for communication with data analysis softwares. The length of each apple roots (recognizable by there brownish color when they age) produced during one interval time (i.e. one week) were calculated for each rhizotron.

We then calculated root elongation rates using the following equation:

$$RER_{t-1,t} = \frac{len_t - len_{t-1}}{P_{t-1,t}}$$

where,  $RER_{t-1,t}$  is the daily root elongation rate (in mm/day) from inventory time  $t-1$  to  $t$ ;  $len_{t-1}$  and  $len_t$  are the lengths of the root  $n$  at inventory time  $t-1$  and  $t$  respectively;  $P_{t-1,t}$  is the period in days between inventory time  $t-1$  and  $t$ .

## Appendix II: Microclimate daily variation

### I. Daily variation of temperature

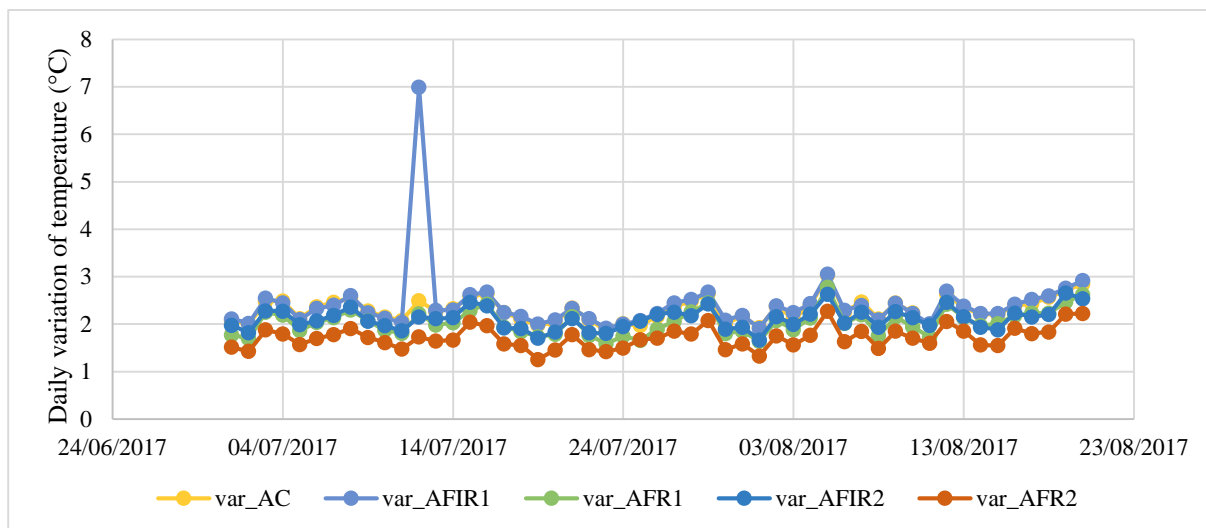


Figure 26: Daily variation of temperature recorded by HMP155 sensors on the plot A1 from the domaine de Restinclières recorded from the 1<sup>st</sup> of July 2017 to the 20<sup>th</sup> of August 2017. There are 5 sensors on the plot, one the open is the control (AC) and the four others are in each agroforestry modality and in the 2 different blocks (AFIR1/AFR1; AFIR2/AFR2) with AFIR: Agroforestry inter-row and AFR: Agroforestry row.

### II. Daily variation in relative humidity

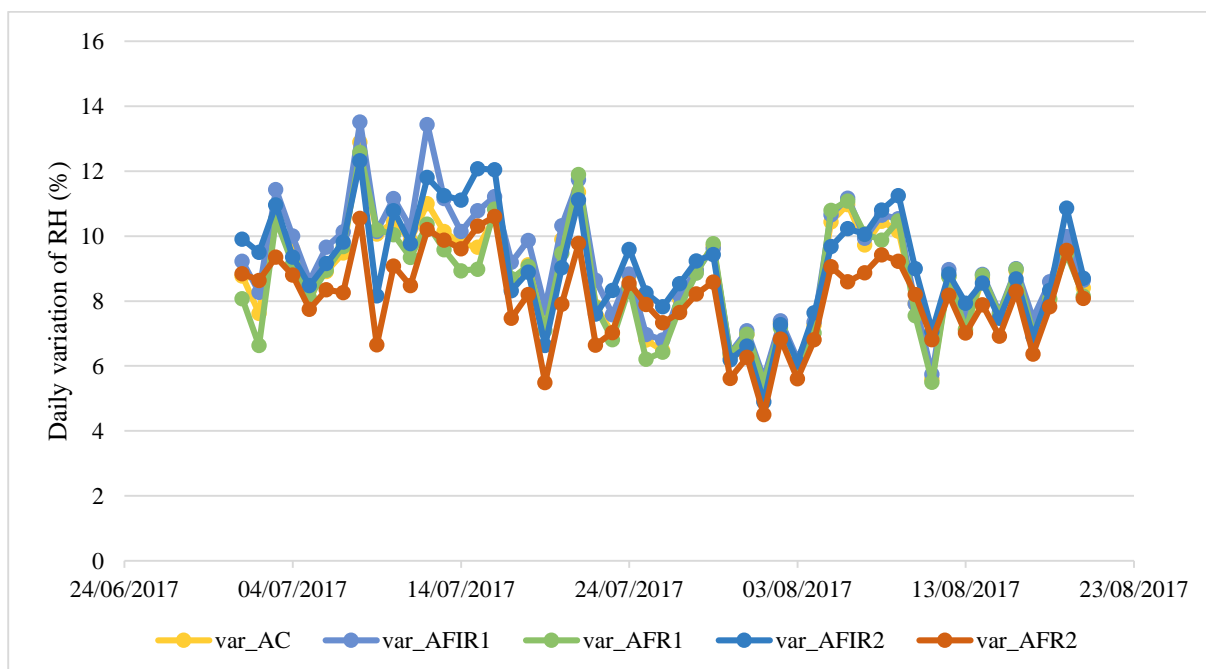


Figure 27: Daily variation of RH recorded by HMP155 sensors on the plot A1 from the domaine de Restinclières recorded from the 1<sup>st</sup> of July 2017 to the 20<sup>th</sup> of August 2017. There are 5 sensors on the plot, one the open is the control (AC) and the four others are in each agroforestry modality and in the 2 different blocks (AFIR1/AFR1; AFIR2/AFR2) with AFIR: Agroforestry inter-row and AFR: Agroforestry row.

## Appendix III: Standardized Major Axis

### I. Original data

#### *SMA results*

Group	n	R2	p	Slope	LowCI	UppCI	Interc	LowCI	UppCI	Ymean	Xmean	H0_b
AF_IR	74	0.652	0.000	0.03487	0.03037	0.04003	2.233	1.629	2.836	6.392	119.270	1.000
	42485.090		0.000									
AF_R	26	0.372	0.001	0.03058	0.02203	0.04246	2.139	0.954	3.324	5.538	111.154	1.000
	10192.651		0.000									
AC	21	0.612	0.000	0.04370	0.03254	0.05867	1.100	-0.632	2.832	6.762	129.571	1.000
	6393.477		0.000									

#### *TEST FOR COMMON SLOPE ACROSS GROUPS*

Grps	N	Slope	LowCI	UppCI	H0_b	X2	p
3	121	0.03557	0.03155	0.03996	1.00000		703.401 0.000
Test statistic:	2.868	p =	0.222				

#### *COMPARISON OF LINES WITH COMMON SLOPE*

Common slope: 0.03557 Grand mean X: 119.314 Grand mean Y: 6.273 Grand mean F: 2.029 Grand mean R: 2.029

Group	n	R2	Interc	YgrandX	XgrandY	Ymean	Xmean	Fmean	Rmean
AF_IR	74	0.652	2.150	6.393	115.920	6.392	119.270	10.634	2.150
AF_R	26	0.372	1.585	5.829	131.799	5.538	111.154	9.492	1.585
AC	21	0.612	2.153	6.397	115.818	6.762	129.571	11.370	2.153

#### *Testing for shifts in elevation between groups using WALD statistic*

df	stat	p	A_com
2	7.882	0.019	na

No significant elevation shift between groups

#### *Testing for shifts along the common slope using WALD statistic*

df	stat	p	A_com
2	11.002	0.004	na

Significant shift along common slope!

Running post-hoc multiple comparisons

Difference, standard error, df, p-value

Group	AF_IR	AF_R	AC
AF_IR	(1.0 )	(1.142 ,6.076 ,1 ,0.014)	(0.736 ,1.816 ,1 ,0.178)
AF_R	(1.142 ,6.076 ,1 ,0.014)	(1.0 )	(1.878 ,9.796 ,1 ,0.002)
AC	(0.736 ,1.816 ,1 ,0.178)	(1.878 ,9.796 ,1 ,0.002)	(1.0 )

p-values only

Group	AF_IR	AF_R	AC
AF_IR	1.0	0.0137	0.1778
AF_R	0.0137	1.0	0.0017
AC	0.1778	0.0017	1.0

## II. Log<sub>10</sub> transformed data

*SMA results*

Group	n	R <sup>2</sup>	p	Slope	LowCI	UppCI	Interc	LowCI	UppCI	Ymean	Xmean	H0_b
AF_IR	74	0.586	0.000	0.5900	0.5075	0.6859	-0.4149	-0.5986	-0.2311	0.797	2.055	1.000
	53.065	0.000										
AF_R	26	0.383	0.001	0.6151	0.4443	0.8514	-0.5112	-0.9253	-0.0972	0.737	2.030	1.000
	9.933	0.004										
TA	21	0.554	0.000	0.7966	0.5812	1.0918	-0.8513	-1.3888	-0.3139	0.824	2.103	1.000
	2.244	0.151										

## Appendix IV: Ordered logit regression

### Week 1

	Value	Std. Error	t value	p value
ModalityAF_IR	-0.1653676	0.2987725	-0.5534901	5.799279e-01
A B	-5.0772294	1.0123649	-5.0152165	5.297378e-07
B C	-4.3766957	0.7243015	-6.0426434	1.516095e-09
C C3	-2.1254637	0.2864671	-7.4195747	1.174970e-13
C3 D	-1.7262634	0.2552443	-6.7631807	1.349947e-11
D D3	-0.6131452	0.2083301	-2.9431418	3.248995e-03
D3 E	-0.1709141	0.2007242	-0.8514870	3.944989e-01
E E2	1.3697446	0.2319009	5.9065943	3.492525e-09
E2 F	2.6902410	0.3606737	7.4589324	8.722637e-14

### Week 2

	Value	Std. Error	t value	p value
ModalityAF_IR	-0.06162504	0.3182506	-0.1936368	8.464603e-01
B C	-5.05052876	1.0128295	-4.9865540	6.146573e-07
C C3	-4.35077406	0.7251063	-6.0001875	1.970898e-09
C3 D	-3.41388314	0.4745892	-7.1933438	6.322346e-13
D D3	-2.24461849	0.3023159	-7.4247453	1.129971e-13
D3 E	-1.39510975	0.2396829	-5.8206475	5.862007e-09
E E2	-0.68849660	0.2132785	-3.2281570	1.245906e-03
E2 F	2.35205911	0.3162398	7.4375812	1.025455e-13

### Week 3

	Value	Std. Error	t value	p value
ModalityAF_IR	0.1400117	0.3039855	0.4605867	6.450952e-01
C C3	-4.2741400	0.7218575	-5.9210300	3.199316e-09
C3 D3	-3.8622993	0.5953163	-6.4878107	8.709260e-11
D3 E2	-2.9872766	0.4057155	-7.3629836	1.798445e-13
E2 F	-1.4912206	0.2447825	-6.0920241	1.114919e-09
F F2	-0.7076308	0.2090147	-3.3855555	7.103435e-04
F2 G	-0.1302528	0.1970219	-0.6611082	5.085429e-01
G H	2.3579395	0.3057061	7.7130933	1.228042e-14

### Week 4

	Value	Std. Error	t value	p value
ModalityAF_IR	-0.150486	0.3730663	-0.4033761	6.866716e-01
C3 F2	-4.403141	0.7311680	-6.0220644	1.722064e-09
F2 G	-3.695577	0.5325743	-6.9390822	3.946555e-12
G H	-1.407942	0.2524790	-5.5764741	2.454424e-08
H I	2.991967	0.4124356	7.2543856	4.034890e-13

### Week 5

	Value	Std. Error	t value	p value
ModalityAF_IR	0.00160706	0.3532905	0.004548835	9.963706e-01
D3 H	-4.33672920	0.7268702	-5.966305121	2.426863e-09
H I	0.89453044	0.2305846	3.879401783	1.047137e-04

### Week 6-7-8

Impossible to run the test because there was only two different phenological stage and that our variable needs at least three categories.

## Appendix V: 2017's growth

### I. Stem length variation coefficient

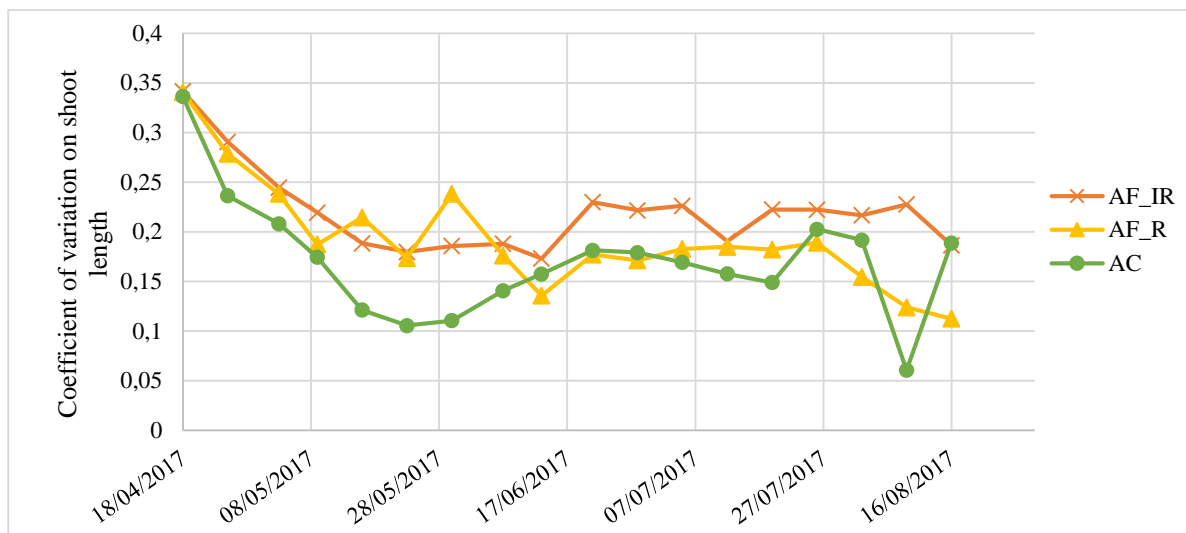


Figure 28: Evolution of the coefficient of variation on shoot length in relation to time of young apple trees (Dalinette cultivar on G202 rootstocks) in a mature agroforestry system (walnut trees) from the experimental site of “domaine de Restinclières” among the different modality (AF\_IR: Agroforestry inter-row; AF\_R: Agroforestry row; AC: Agricultural control).  $C_v = \sigma/\mu$ .

### II. Number of leaves

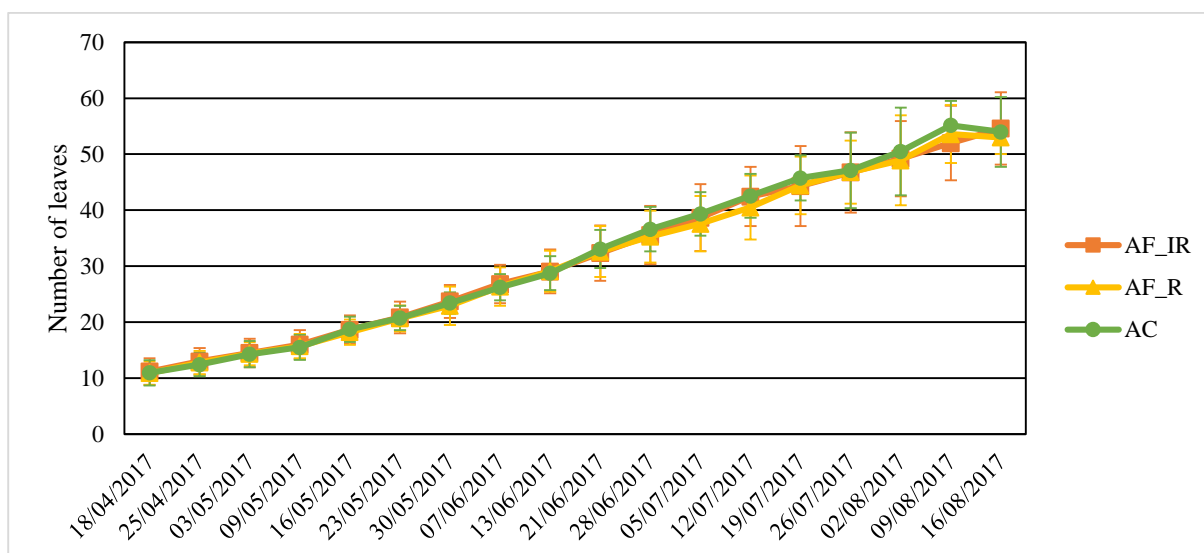


Figure 29: Number of leaves on the shoot grown during the second year of two-year-old apple trees (Dalinette cultivar on G202 rootstocks) in a mature agroforestry system (walnut trees) from the experimental site of “domaine de Restinclières”. The number of leaves was incremented each week for 18 weeks. We used an ANOVA when possible or a Kruskal-Wallis rank test ( $\alpha = 0.01$ ) to highlight significant differences between our three modalities: AF\_IR: Agroforestry inter-row; AF\_R: Agroforestry row; AC: Agricultural control.



### III. Statistical results on 2017's growth

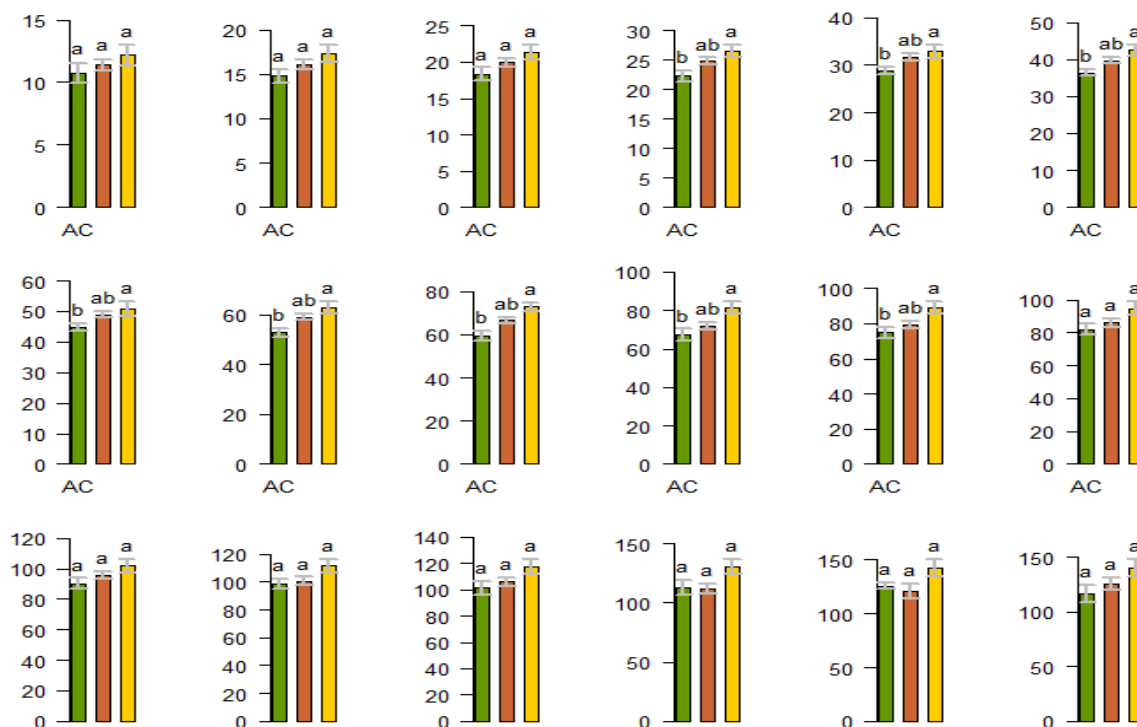


Figure 30: Shoot length statistical results from week 1 (top left) to week 18 (bottom right). Modalities: Green = AC, Orange = AF\_IR, Yellow = AF\_R. The statistical analyse was made using a Kruskal-Wallis test rank ( $\alpha = 0.01$ ).

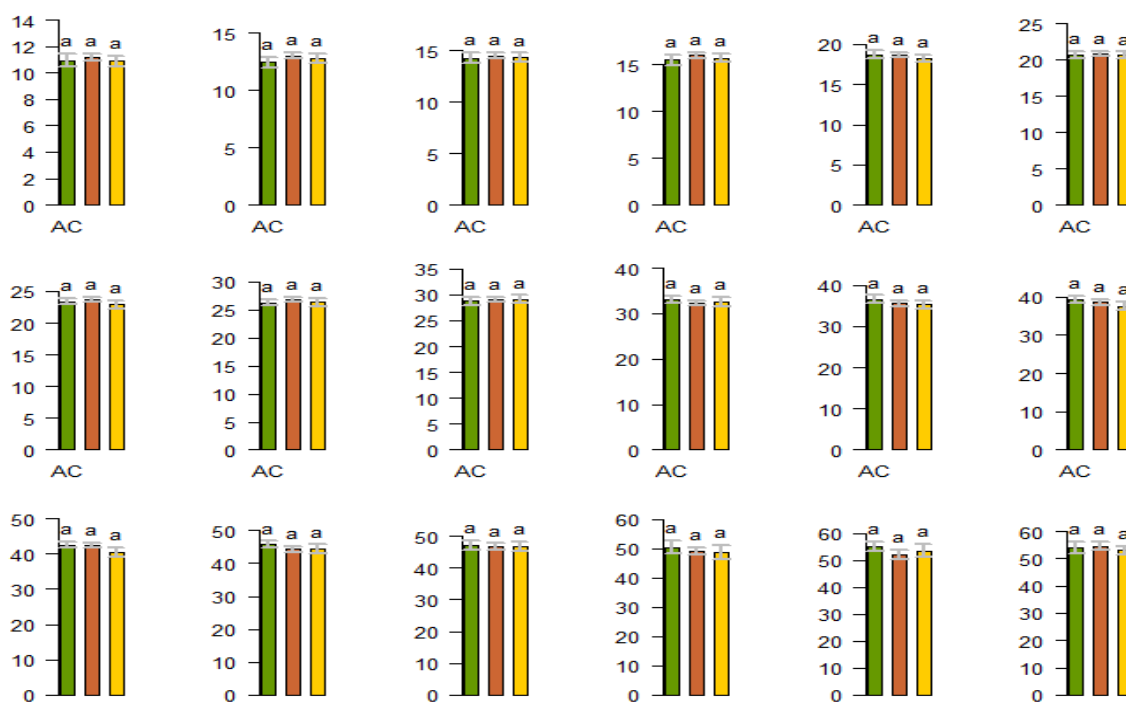


Figure 31: Number of leaves statistical results from week 1 (top left) to week 18 (bottom right). Modalities: Green = AC, Orange = AF\_IR, Yellow = AF\_R. The statistical analyse was made using a Kruskal-Wallis test rank ( $\alpha = 0.01$ ).

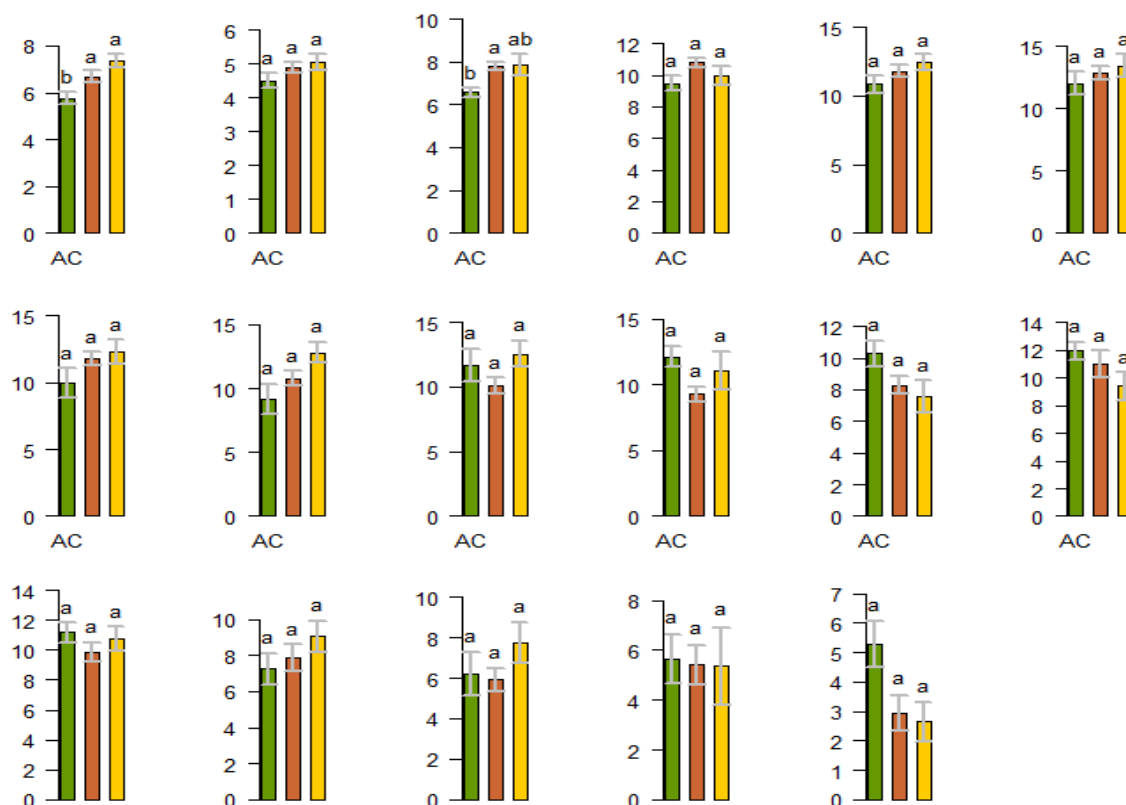


Figure 32: Growth rate statistical results from week 1 (top left) to week 18 (bottom right). Modalities: Green = AC, Orange = AF\_IR, Yellow = AF\_R. The statistical analyse was made using a Kruskal-Wallis test rank ( $\alpha = 0.01$ ).

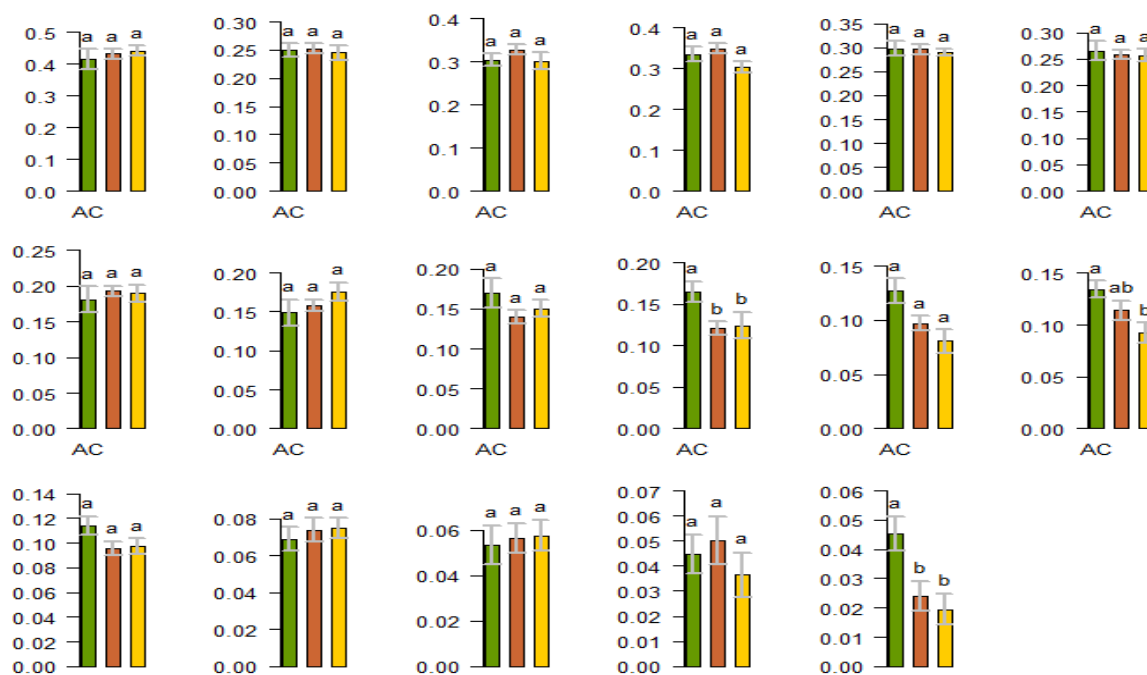


Figure 33: Relative growth rate statistical results from week 1 (top left) to week 18 (bottom right). Modalities: Green = AC, Orange = AF\_IR, Yellow = AF\_R. The statistical analyse was made using a Kruskal-Wallis test rank ( $\alpha = 0.01$ ).



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## Abstract

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Contemporary agriculture has to face new scientific and societal challenges (IAASTD 2009). Recent studies have shown that humanity will have to double agricultural production to meet the demand in 2050 (Ray et al. 2013). But agriculture needs to increase its production while addressing environmental concerns. Different solutions have been proposed in order to answer these challenges including new practices more resilient to external pressure (climatic disturbances, new diseases, economic crises) and more efficient (Foley et al. 2011).

Agroforestry is the association, on a plot, of perennial and annual crops and possibly animals (Dupraz & Liagre 2011). Agroforestry systems (AFS) in temperate climate are mainly composed of two layers: the tree layer and the herb layer. However, an original timber-tree-based AFS could be enriched with fruit-trees in an intermediate vertical stratum. In such context, the interest but also the challenges of timber- and fruit-tree based AFS lie in: (i) fruit production, (ii) microclimate and plant ecophysiology and (iii) biocontrol of pests and diseases (Lauri et al. 2016). The apple-agroforestry experiment (Growing AgroForestry systems with Apple, GAFA) was set in 2016, combining hybrid walnut trees planted in 1995, apple trees planted in March 2016 and 5-year-old Lucerne.

This work focuses on the effects of the distance between apple trees and walnuts, inducing presumably a gradient of competition, on various architectural and morphological aboveground features of the apple trees. The AFS reduced incoming light and global radiation but acted as a buffer on temperatures. These modification on the aboveground environment of the AFS induced a buffer effect on water potential daily evolution ( $\Psi$ ) as well as different growth strategy of the apple tree in their second year. There was a difference in the AF apple trees stem geometry (higher slenderness and lesser tapering), architecture (less ramifications), and morphology (higher leaf area and specific leaf area).

**KEYWORDS:** Agroforestry, microclimate, apple trees, architecture.

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## Résumé

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L'agriculture contemporaine doit faire face à de nouveaux défis scientifiques et sociétaux (IAASTD 2009). De récentes études ont montré qu'il sera nécessaire de doubler la production agricole mondiale pour répondre à la demande en 2050 (Ray et al. 2013). De plus, l'agriculture doit limiter ses externalités négatives. Le développement de nouveaux agrosystèmes plus résilients face aux pressions externes (réchauffement climatique, maladies, crise économique) et plus efficaces dans leurs utilisations d'intrants fait partie des solutions avancées (Foley et al. 2011).

L'agroforesterie consiste en l'association, sur une même parcelle, d'essences végétales pérennes et annuelles et éventuellement d'animaux (Dupraz & Liagre 2011). Les systèmes agroforestiers (AFS) en climat tempéré sont majoritairement composés de deux strates : la strate arborée et la strate herbacée. Cependant, un système agroforestier à base de bois d'œuvre peut être enrichi d'une strate verticale intermédiaire composée d'arbre à fruits. Les intérêts et les défis d'un tel système réside dans : (i) la production de fruit, (ii) le microclimat et l'écophysologie des plantes et (iii) le biocontrôle des ravageurs et maladies (Lauri et al. 2016). L'expérimentation a été mise en place en 2016 combinant des noyers hybrides plantés en 1995, des pommiers plantés en mars 2016 et une luzernière de 5 ans.

Ce rapport s'intéresse aux effets de la compétition aérienne avec les noyers sur l'architecture et la morphologie des pommiers. L'AFS réduit la lumière incidente et le rayonnement global en plus de tamponner les variations quotidiennes de températures. Ces modifications du microclimat induisent une moindre variation quotidienne du potentiel hydrique ( $\Psi$ ) ainsi que différentes stratégie de croissance des pommiers. Je montre ici des effets du contexte agroforestier sur la géométrie des pousses du pommier (élancement plus important et conicité moins élevée en agroforesterie), sur son architecture (moins de ramification axillaire) et sur sa morphologie foliaire (surface et surface foliaire spécifique plus grandes).

**MOTS CLES :** Agroforesterie, microclimat, pommier, architecture.