



UNIVERSITÀ DEGLI STUDI DI PADOVA

Dipartimento di Agronomia Animali Alimenti Risorse Naturali e
Ambiente

Corso di Laurea Magistrale in SCIENZE E TECNOLOGIE AGRARIE

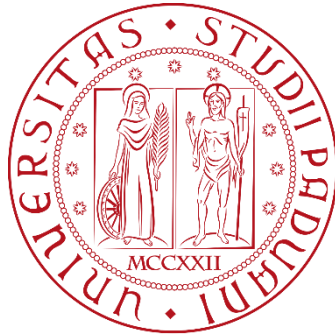
***Screening of crop varieties for agroforestry farming: the case
of durum wheat in organic olive orchards in southern France.***

Relatore: Ch.mo Prof. Teofilo Vamerali

Correlatore: Dr. Cristian Dal Cortivo

Laureanda: Anna Panozzo

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Ai miei nonni

Silene e Albino

Imelda e Giuseppe

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ABSTRACT

Agroforestry, defined as integrating trees and shrubs with annual crop production, is receiving considerable attention as sustainable land use to improve resilience to climate change. Agroforestry model is widely cited for its potential to address various on-farm adaptation needs in developing countries, therefore there is the need of experimental results and references in Europe. In this study, it was investigated a durum wheat/olive tree agroforestry model in the Mediterranean climate conditions of Southern France under organic farming system.

To do that, it was studied two agroforestry treatments where the olive tree canopies intercepted the 33 % of PAR in one case and the 55% of PAR in the other case, due to different canopy size. The two agroforestry treatments were compared with a durum wheat control system, grown in full sun conditions. the following five topics were investigated:

The impact of agroforestry on the microclimate and the edaphic environment experienced by crops. Further to the reduction in solar radiation reaching the understored crop, trees cause a buffer effect in the daily temperature cycle: temperature recorded was lower during the day time and higher during the night time, compared to full sun conditions. Wind speed was significantly reduced by trees.

The impact of Agroforestry on durum wheat phenology. The phenological development was delayed by 2 to 9 days within agroforestry treatments. A variability was seen between wheat varieties.

The impact of Agroforestry on durum wheat yield, yield components and morphology. Yield was decreased by 47% (till 55% in the most shaded treatment). The number of grains per spike was the most affected yield component, with an average reduction of 45 %. Plant height and spike size were decreased within agroforestry systems, but the distance between the flag leaf to the spike in the culms tended to increase under shading. The protein content was increased by 12 % on average in shaded treatments compared to not shaded one.

Screening durum wheat varieties for agroforestry: searching for an appropriate test. There is the need to provide farmers with varieties adapted to agroforestry. The same varieties tested in the field were sown in pots placed inside a greenhouse and artificial shade was created with a cover shelter. The percentage reduction of certain traits between the greenhouse-control treatment and the greenhouse-shade treatment were similar to the one observed between the field-control treatment and the field-shade treatment.

The impact of associated field crops on organic olive orchards production. The purpose is to implement a crop within an organic olive orchard. The durum wheat intercropped determined an increase of 7 % in the olive tree productivity over a 3-year period compared to the “Forest Control (natural grass in the inter-rang). Considering the production costs and the market prices of the southern France context, the potential additional income arising from this increased production and from the durum wheat sold, is of +555 euros/ha.

INTRODUCTION

Context

This report presents the results of the internship I realized from March 2017 till September 2017 at INRA in Montpellier. This internship was part of a European research project called Agforward, AGroFOREstry that Will Advance Rural Development.

Agroforestry is a land use where trees and shrubs are integrated with annual crop production (Mbow et al. 2014) in the same field with the aim of a more sustainable and more efficient use of available resources. AGFORWARD is a four-year research project started in 2014 and will finish in December 2017. It involves over 23 universities, research and farming organisation across Europe and aims to evaluate innovative agroforestry design and practices, and to promote the wider adoption of appropriate agroforestry systems in Europe.

In the frame of this project, the INRA in Montpellier implemented an agroforestry system in 2014 and carried on several experiments aiming to respond to the project's objectives. Within the different topics, it was decided to be part of the Working package number 4: "Agroforestry for crop production". Therefore, from 2014 till 2017 a durum wheat crop was implemented in the interrows trees into farming systems (Chahan et al., 2010), but the idea beside this agroforestry system was, on contrary, to introduce crops into trees systems.

The objectives defined in the study of this durum wheat/olive tree agroforestry model were:

- To study the impacts of trees on the crop;
- To identify the durum wheat varieties suitable to be cultivated within an olive tree orchard in organic farming;
- To evaluate the appropriateness of a pre-breeding test in pots looking for shade-tolerant varieties;
- To valorize the local olive orchards through the implementation of a crop in the interrow.

As light is likely to be the principal limiting resource for the understorey crops (Artru et al. (2017), responsible of the yield reduction, several authors concluded that the success of agroforestry depends on the selection of shade-tolerant species. But, before selection, there is the need to study this farming system in its complexity and to monitor the impacts of this example of association on the crop chosen and on the trees involved.

In order to evaluate this durum wheat/olive tree agroforestry model, every year a great number of data were collected. Before me, two other students (“BTS” formation) carried on their internship in this experiment site for the 2014/2015 and for the 2015/2016 crop seasons. They collected data, started to analyse them and then wrote a report. For the last year of field experiments (season 2016/2017) the researcher responsible of this project at INRA in Montpellier was looking for a Master student which could carry on the same experiments as done in the previous years, which could add some complementary experiments during his internships and which would have the charge to analyse all the data and valorise them at the end of the project.

I took part of this project, then, in the most important year. The project will end in December 2017 and the aim is to disseminate the results obtained after the 4-year experiment through publications. It was decided, then, to be oriented to publications from the beginning of my internship, in March 2016. This is the reason why this report will not present the structure which normally has the “rapport de fin d’études”.

The results of the internship will be presented in the form of scientific articles and in English language. Moreover, as the experiments focused mainly on five topics over the Agroforestry model studied, this report will present the results obtained divided in five chapters:

1. The impact of Agroforestry on the microclimate and the edaphic environment experienced by crops.
2. The impact of Agroforestry on durum wheat phenology.
3. The impact of Agroforestry on durum wheat morphology, yield and yield components.
4. Breeding test for agroforestry: looking for an appropriate test.
5. The impact of associated field crops on organic olive orchards production.

The impact of Agroforestry on the microclimate may have an effect on crop phenology, and changes on phenology can affect crop yield. Then, the crop yield reduction highlights the need of breeding programs looking for shade tolerant varieties, and thus the pertinence of a breeding test in pots was investigated in the chapter n. 4. Finally, as the idea of this project was to introduce crops into trees systems, the fifth paper presents the impacts of the durum wheat crop associated on the organic olive orchard production.

This division in five chapters, therefore, aimed to present the results in a logical order but might be a limitation as every part is very connected to the others.

During my internship, I was doing all the experiments that will be described in the five articles and, thus, a great part of the time was dedicated to data collecting. Moreover, I was glad to add the Mycorrhizal analyses (describes in the first article) and the analyses of composition of proteins (described in the third article) which were not done in the previous years.

From the beginning of my internship, I was studying the literature with the aim of these five articles. The time to analyse all data collected was short, but enough to show the complexity of the impacts of Agroforestry as this report wants to do. As the responsible of the project proposed me to stay 3 months longer, now my goal is to go on with analyses and to integrate the results from the previous years to valorise the knowledge achieved before the end of the project in December 2017.

In this report the articles from 1 to 4 present the results from the data I collected during the last year of the project; on the contrary, the last chapter presents the data collected during the 4-years period to show the evolution of the olive trees production after the implantation of the under stored durum wheat crop.

Study site

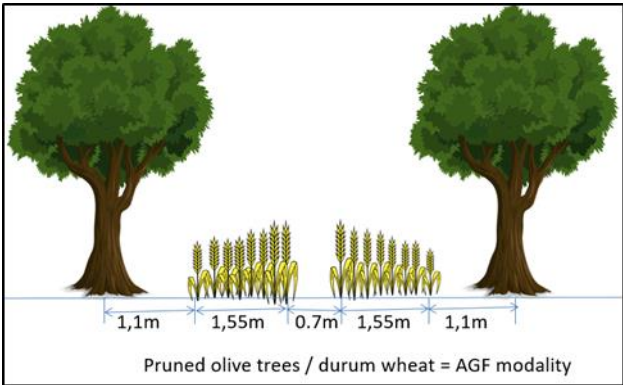
The experiments were conducted at INRA station DIASCOPE in Manguio (43° 35' N, 3° 45' E). The climate is sub-humid Mediterranean with a yearly average temperature between 14.5 and 15 °C. The level of sunlight (7 h 22 min per day in average) is one of the higher in France (french mean= 4 h 46 min). The annual precipitation level is about 750 mm with a high heterogeneity in rainfall patterns. The number of rainy days is low (less than 60 per year on average).

The field trial was composed by 3 treatments: 2 agroforestry systems and a control, as shown in the Figures below.

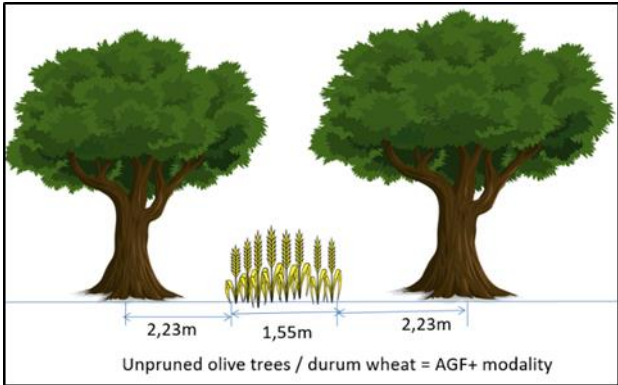
AGF, the first agroforestry treatments, is an olive orchard where trees are spaced 6 m x 6 m and they have been yearly pruned, thus it could host 2 durum wheat plot lines.

AGF+, the second agroforestry treatment, is an olive orchard where trees have never been pruned in the frame of a previous project investigating the architecture of the canopy. While the distance between rows is the same as in AGF, the canopy size is greater. It represents an extreme condition of shade and it could host one line of durum wheat plots.

These two treatments have been investigated in comparison with a **control**, where durum wheat has been sown in open field conditions, without the presence of olive trees.



AGF



AGF +



CONTROL

Pedological hole dig in the soil within the AGF treatment let to assess the position of the tree roots. Olive tree roots seem to grow toward the deep soil layer and not toward the durum wheat crop in the inter-row, thus indicating a deep ground soil available for root growth of wheat in this site.

The main originality, compared to classical agroforestry systems is the specificity of the olive tree species. Indeed, the examples found in literature concern mainly deciduous trees as Pawlonia trees or walnut trees (Li et al (2008); Dufour et al. (2013)). When the crop is associated with deciduous trees, it reaches its maximum LAI before tree leaf development in spring get to the maximum LAI and affect the crop with its shade. In the case of this study, olive trees are evergreen species and this leads to shade effects on the understored crop for the whole period of its growth.

Additionally, the distance between the olive tree rows is very short (6m) compared to classical agroforestry designs (15 to 25 m). This short distance leads to a high light reduction for the plants located between tree rows and for part of them directly under the foliage crown.

I. IMPACT OF OLIVE TREES ON THE MICROCLIMATIC AND EDAPHIC ENVIRONMENTS OF THE UNDERSTOREY DURUM WHEAT CULTIVARS.

1. Introduction

Negative impacts of climate change on crop production have been widely investigated (Nguyen et al. 2013, Lobell et al. 2008; Parry and Carter 1985; Pautasso et al. 2012). The increase of the average annual temperature and frequency of extreme temperatures (IPCC, 2014) are the main critical factors of climate change process. In the Mediterranean area, increased temperature mainly increased evapotranspiration and, therefore, crop water requirements, with modifications of plant phenology (Rao et al. 2007, Chmielewski et al. 2001).

Agroforestry, defined as integrating trees and shrubs with annual crop production (Mbow et al. 2014), is receiving increasing attention for its potential role in improving resilience to climate change. Trees provide shade with their canopy and shelter thorough windbreak effect. There are several hypotheses found in the literature on the role of trees in microclimate buffering and regulation of water flow. As stated by Sanchez (1995), among 16 biophysical agroforestry hypotheses, when water is limiting, trees can provide mitigation action. They reduce evaporation water losses and conserve soil moisture. Moreover, slowing the movement of air, they are stated to reduce air temperature and maintain a more moderate microclimate for crop growth (Schoeneberger et al. 2012).

Trees also improve the soil structure by increasing fertility and help prevent soil erosion. Probing root growth breaks up the soil, which creates spaces for storing air and water. Tree roots improve drainage because each root acts as an underground water channel to help water penetrate the soil. Some tree roots add nutrients to the soil, which naturally fertilizes the surrounding plants. But to catch these nutrients, the understorey plant crop can be help by the way of fungi and especially the arbuscular mycorrhizal (AM) fungi. These fungi are recognized as an essential component of sustainable agricultural ecosystems (Jefferies et al. 2003; Vázquez et al. 2000). AM isolates varied in responsiveness, establishment and colonization, with plants depending on edaphic factors (Fabig et al., 1989). The importance of maintaining active populations of AM fungi in agroforestry soils in order to sustain crop productivity has also been demonstrated (Sieverding and Leihner (1984); Dodd et al. (1990)). More recently, Arihara and Karasawa (2000) have shown that maize yields were better, and mycorrhizal fungus colonization higher, in fields cultivated after other mycorrhizal crops, rather than in maize cultivated after non-mycorrhizal crops.

Despite the many hypotheses of tree impact on the understorey crop, to date, few are the studies which demonstrated it experimentally in north Mediterranean area. Results available in literature concern mainly tropical regions and most often legume trees. Coffee agroforestry systems have been shown to benefit the crop understored by affecting the balance of water in the system (Lin et al. 2010). Studies carried on in Mexico assessed that the amount of shade cover was directly related to the mitigation of variability in microclimate and soil moisture. Alley-cropping with *Leucaena leucocephala*, a typical tropical tree species, has also been investigated. In India, alley-cropping of sorghum and cowpea with this tree species, induced competition for moisture which reduced yield from 30 to 100% (Singh et al. 1989). Furthermore, agroforestry model is widely cited as a key solution to increase soil fertility in developing countries, especially South America (Pinho et al. 2012) and Africa (Sanchez et al. 2002), and for its potential to address various on-farm adaptations needs (Rao et al. 2007). However, there is a need of experimental results and references in Europe on the impact of non-leguminous trees on the microclimate and the soil fertility of the understored crops.

In this paper, we investigate an alley-cropping model, based on olive trees and an understored durum wheat cultivation in Mediterranean climate conditions. In an experimental site of a region in the south of France, sustainability of an olive orchard agroforestry system has been investigated during 4 years. In order to evaluate the potential role of agroforestry farming system in reducing vulnerability of crops to uncertain and shifting environments (Noordwijk et al., 2011), this work aims at assessing the:

- Shading effect of trees: the impact on growing crop under agroforestry system is a key issue;
- Microclimate modification due to windbreak and shade effects: impact on airflow, air temperature and humidity;
- Water availability: does trees provide additional water to crops and increase water use efficiency?
- Soil fertility: what is the impact of trees on soil NO_3 and NH_4^+ contents?
- Does the agroforestry system promote beneficial biological interactions between micro-organisms and plant species, especially arbuscular mycorrhizal fungi (AMF) in roots?

2. Materials and methods

2.1 Study site

The experiments were conducted at the INRA station DIASCOPE in Maugeio (43° 35' N, 3° 45' E). The climate is sub-humid Mediterranean with a yearly average temperature between 14.5

and 15 °C. The level of sunlight (7 h 22 min per day in average) is one of the higher in France (french mean= 4 h 46 min). The annual precipitation level is about 750 mm with a high heterogeneity in rainfall patterns. The number of rainy days is low (less than 60 per year).

2.2 Experimental design

25 genotypes of durum wheat (*Triticum turgidum* sub. *durum*) were sown (sowing density = 350 seeds/m², distance between wheat rows=0.16 m) each year around mid-November (just after olive harvesting) in 3 experimental conditions (treatments): 2 olive orchards, one never pruned (AGF+) and one yearly pruned (AGF), and an open field without trees (Control).

The genetic variability considered in the test for durum wheat has been reported in Table 1. 14 of the 25 cultivars are durum wheat varieties and the other 11 are populations. Among pure lines there were modern well-known varieties (Clovis, Claudio, Dakter and Surmesur), a recently selected variety for organic farming (LA1823), and ancient varieties coming from the genebank maintained by INRA at Clermont-Ferrand.

In each treatment, a randomized block design was implemented with 2 replicates per genotype. Each plot consisted in 1.55-m width and 10-m long. Each treatment then hosted 50 plots of Durum wheat in an annual rotation with legumes crops.

In the intercropping treatment, coded AGF (figure 1-right), durum wheat plots were cultivated in an olive orchard where olive trees have been regularly pruned from 2012. This orchard has been planted in 2002 and is composed of 8 rows (6 m x 6 m) in a 0.5 ha area. Olive trees are different clones of Picholine, Verdale-de-l'Hérault and cross between both. They were oriented along the long axis north-west south-east of the plain. The 6 m inter-row space allowed hosting side-by-side 2 durum wheat plots. Between these two neighbors plots there were 0.7 m of no cultivated ground soil and between olive trees trunks and durum wheat plots there were a 1.10 m space covered by permanent natural grass.

The other intercropping treatment, coded AGF+ (Figure 1-left), took place in an olive orchard planted in 2002 as well, located closely (20 m west) to AGF treatment. In this orchard, olive trees (clones coming from a cross between Arbequine and Oliviere) were never pruned. Even if the inter-rows were also 6 m, the canopy size was larger than in the AGF orchard, due to no pruning. Consequently, only one plot width was possible to be sown in each inter-row.

The Control treatment (open field without any tree) coded C, was located also closely to the 2 others treatments. It hosted also 25 genotypes in a randomized 2-replicated block design.

Table 1. List of the 25 durum wheat genotypes tested: the code used in all papers to identify them, their names and type (VAR=VARIETY, POP=population). Among varieties, 8 were modern well-known varieties, 6 were ancient varieties coming from the genebank maintained by INRA Clermont-Ferrand

variety code	variety name	type	VAR characteristics
1	LA1823	VAR	modern
2	Clovis	VAR	modern
4	2007D023.655	VAR	modern
6	2007D003.109	VAR	modern
9	2007D010.255	VAR	modern
11	Pop Algérie 1	POP	
12	Pop Algérie 2	POP	
13	Pop Algérie 3	POP	
14	Pop F2 + lég Salernes	POP	
16	Pop F2 + lég Ampus	POP	
22	Pop F3 + lég Mauguio	POP	
43	RG 425	VAR	ancient
45	RG 137	VAR	ancient
55	Pop_PMG	POP	
56	Pop_PROT	POP	
57	Pop_Sécheresse	POP	
58	Pop_HR	POP	
59	pop NirS	POP	
77	El_Khroub_06	VAR	ancient
79	El_Khroub_10	VAR	ancient
100	Claudio	VAR	modern
101	Dakter	VAR	modern
102	Surmesur	VAR	modern
266	RG 266	VAR	ancient
534	RG 534	VAR	ancient

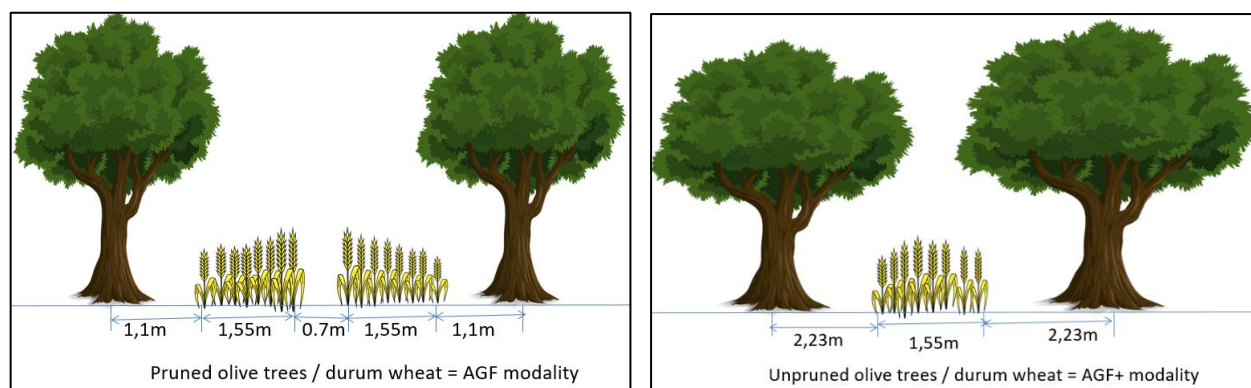


Figure 1 Scheme of olive trees/durum wheat intercropping for AGF and AGF+ treatments.

2.3 Collected data

Microclimate parameters were recorded from the day of wheat sowing to the day of wheat harvesting at the end of June. A Meteo-France weather station - model “CLIMATIK” - located in the Mauguio INRA center, very close to the experimental field, provided daily air temperature, air humidity, rainfall, global radiation and wind speed. Beside this permanent weather station, different tools recording microclimatic parameters were placed in the 3 treatments at wheat sowing time and then removed just before harvesting. To avoid the possible influence of wheat genotype factor, the pool of microclimate tools was placed in each treatment in the middle of the plot of LA1823 genotype, block 1.

Sensors situated in AGF and AGF+ treatments were providing PAR, air and soil temperature, wind speed and soil water content. Sensors placed in the C treatment were providing air and soil temperature and soil water content. Considering PAR and wind speed parameters of control treatment, data recorded in the “CLIMATIK” weather station were collected for the analysis. Additionally, sensors recording air temperature and humidity for AGF and C treatment (sensors located in CLIMATIK meteo station) were used in the analysis.

2.4 Shade measurement

Solar radiation was recorded in the 3 treatments through SKS 1110 PYRANOMETER for global radiation (Skye Instruments LTD, linearity error -0.2%, working range -0 – 500 w/m²) and Pyranometer SP-LITE for PAR (Campbell Scientific Ltd, linearity error <10%, working range 0.4 - 1.1 μm). AGF and AGF+ treatments were provided by 3 global radiation and 3 PAR sensors each one. 2 were positioned in the middle of an inter-row, spaced 6 m one from the other along the inter-row. The third one was in the inter-row next to the first one, at half-distance between the 2 other sensors, as shown in Figure 2. Radiation parameters were recorded every 15 minutes. For control treatment, data collected each hour from sensors located in the CLIMATIK station were used. Sensor model was the same as in the other treatments. Output data of global radiation and PAR for the 3 treatments were recorded in Joule/cm² (tools for C) or in μmol m⁻² s⁻¹ (tools for AGF and AGF+), then converted in W m⁻². To measure the solar radiation in line with the tree crown, one of the 3 PAR sensors of AGF system was moved from the middle of the alley to the olive tree row at one meter distance from the trunk from 20/04/2017 to 28/04/2017.

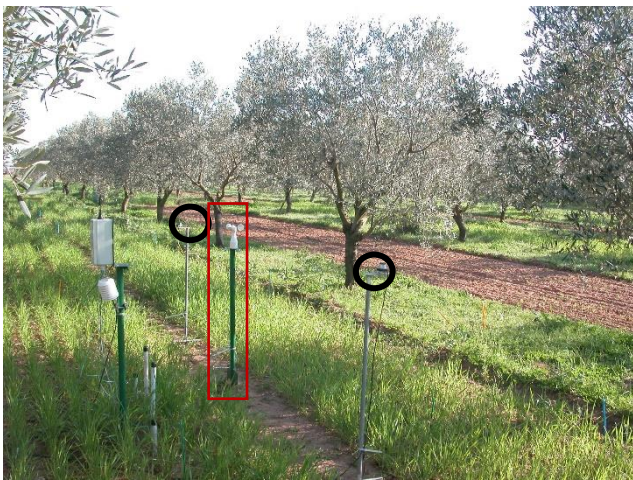


Figure 2 Picture taken in the AGF treatment showing the position of the two PAR sensor placed in the middle of the same inter-row (on the left) In red is shown the position of the anemometer. Scheme of the position of the three PAR sensor in AGF treatment; in light blue the code of the wheat cultivars for each plot (on the right).

2.5 Microclimate parameters

2.5.1 Air temperature and relative humidity

Air temperature and relative humidity at 1 m height was measured only for AGF and C treatments, but not for AGF+. For AGF, the data came from sensors located directly in the field whereas for C treatment, data came from permanent Climatik station. Data were logged by HMP60 probes, through INTERCAP capacitive RH chip. Manufactured by Vaisala, it measures air temperature within the range from -40° to 60°C , and relative humidity (RH) within the range 0-100%. For AGF treatment, average air temperature and average relative humidity were recorded every 15 minutes, then they were averaged hourly. For C treatment, output data are directly recorded hourly.

2.5.2 Wind velocity

A 3 cups anemometer (Campbell Scientific Ltd, linearity error $<1.5\%$, working range 0 – 45 m/s) was placed in each treatment. Average wind velocity and maximal wind velocity, both in m/s, were recorded every 15 minutes for AGF and AGF+ and hourly for C treatment. The anemometer was placed in the middle of the inter-row at 1 m height as shown in Figure 2.

2.6 Edaphic parameters

2.6.1 Soil moisture content

Four soil moisture sensors were placed in each treatment in the plot of LA1823 durum wheat variety at different depths: 30, 60, 90, 110 cm. Probes were WATERMARK Model 200SS (manufactured by IRROMETER company, working range 0 – 239 KPa. The watermark system correlates the resistance of the current applied in each probe to KPa of soil water tension. Data were recorded every 15 minutes.

2.6.2 Soil sample analyses

To understand the impact of crops on the soil quality, 13 georeferentiated points have been sampled between trees each year in February. Each sample has been collected at 3 depths, 0-30, 30-60 and 60-90 cm, and total amount of N, NO₃ and NH₄ content have been determined for each sample by laboratory Auréa (<https://www.aurea.eu/>).

2.6.3 Mycorrhizal colonization.

Mycorrhizal analysis was performed on 6 durum wheat pure lines chosen among modern varieties (1, 4 and 101 in Table 1) and among ancient varieties (43, 45 and 266 in Table 1). In each field treatment (AGF+, AGF and C) and for the 6 varieties, all the plants over the width of 40 cm were removed on 26/04/2017 (wheat stage: BBCH-50) by means of a fork. Plants and the mycorrhizal soil (attached to roots) were collected in plastic bags and stored at – 4 °C. Mycorrhizal analyses were done the week after at LSTM (Laboratoire de Symbioses tropicales et méditerranéennes) of CIRAD Baillarguet. Plant roots of each sample were cleaned from soil and 1 cm was cut in the central part of the root length. 270 root fragments of 1 cm (obtaining 3 repetitions of 90 root fragments) were analysed for each variety and treatment. Root mycorrhiza were coloured and observed as stated by the MO-M-C-04 protocol (Delteil et al., 2016), version A. Fragments were observed under microscope and rated according to the range of classes indicated in the protocol. Data were then proceeded through Mycocalc software (<http://www2:dijon.inra.fr/Mycocalc-prg.html>), and following parameters were calculated: frequency and intensity of mycorrhiza colonization in the root system, intensity of the mycorrhizal colonization in the root fragments, arbuscular abundance in mycorrhizal parts of root fragments, arbuscular abundance in the root system.

3. Results

3.1 Solar radiation

PAR and treatments. There was a great difference between treatments as regards the available photosynthetic active radiation that reached the crop. Considering the entire period from sowing to harvesting, the measures recorded in control treatment showed an average of 381 PAR $\text{m}^2 \cdot \text{s}^{-1}$ and a max value of 2044 PAR $\text{m}^2 \cdot \text{s}^{-1}$ (figure 3). In AGF treatment, the mean around 256 PAR $\text{m}^2 \cdot \text{s}^{-1}$ with a max = 1869, showed that almost 33 % of the PAR radiation was intercepted by trees and didn't reach the crop. For AGF+ treatment, this percentage of interception was around 55 % (mean=170 PAR $\text{m}^2 \cdot \text{s}^{-1}$, max = 1857).

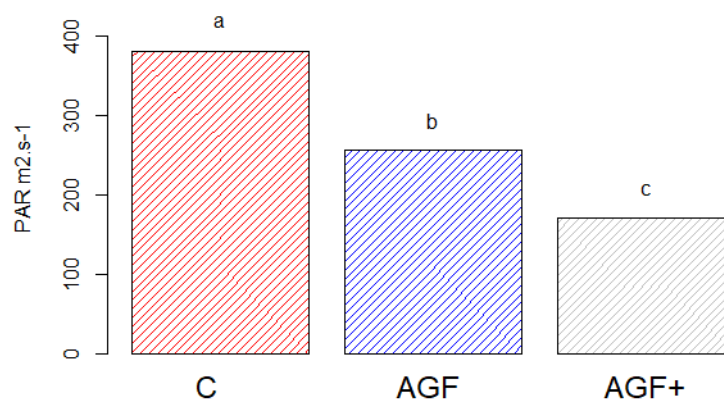


Figure 3 : PAR ($\mu\text{mol m}^{-2}\text{s}^{-1}$) reaching durum wheat canopy in the 3 systems. PAR data recorded hourly and averaged from sowing to harvesting.

In details, across the growing period (Table 2), the reduction in PAR received in AGF treatment compared to full sun conditions (Control) was 38 % during stem elongation, 29% during anthesis and 30 % during maturity. Olive trees in AGF+ treatment intercepted 52% of PAR radiation during stem elongation, 48% during anthesis and 62% during maturity.

Table 2 Average PAR data recorded hourly for the 3 growth periods. Mean's with different letters are significantly different according to Tukey's HSD within same period.

	Treatment		
	C $\mu\text{mol m}^{-2}\text{s}^{-1}$	AGF $\mu\text{mol m}^{-2}\text{s}^{-1}$	AGF+ $\mu\text{mol m}^{-2}\text{s}^{-1}$
Germination – stem elongation	249,92 a	155,22 b	117,36 c
Stem elongation – anthesis	518,11 a	365,41 b	267,14 c
Anthesis - maturity	636,76 a	448,71 b	245,15 c

Moreover, by detailing the dynamics of PAR over the central part of the day, from 11 a.m. to 3 p.m. (Figure 4), the reduction in PAR received in AGF treatment compared to full sun conditions (Control) was averagely 22% (from 1099 in C to 855 $\mu\text{mol m}^{-2}\text{s}^{-1}$ in AGF). PAR radiation reaching the wheat in AGF+ treatment during this same period was decreased by 61 %.

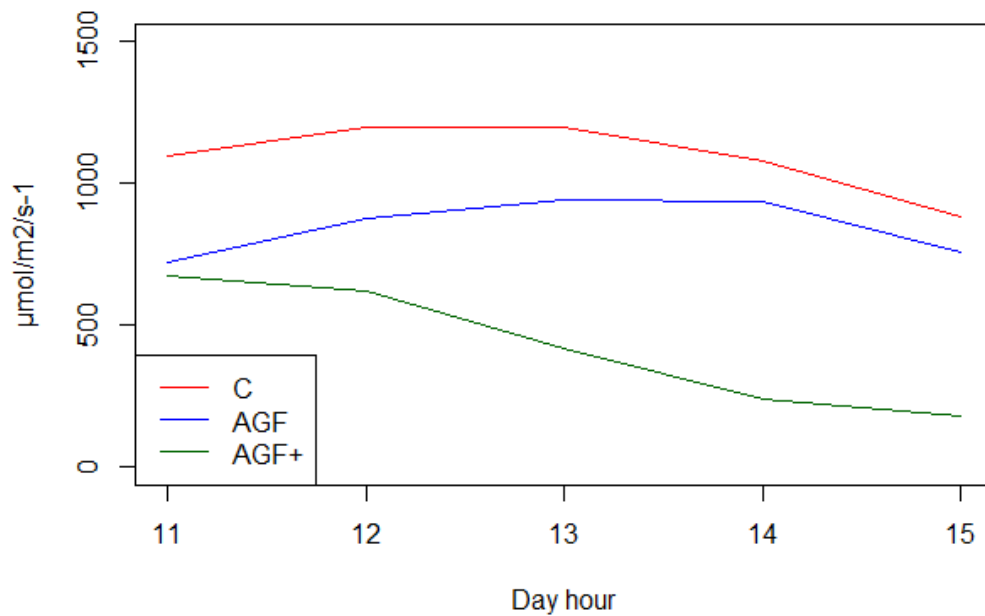


Figure 4 Average PAR $\text{m}^2 \cdot \text{s}^{-1}$ reaching the three treatments in the central hours of the day from sowing till harvesting : from 11 am till 3 pm at the center of the alley.

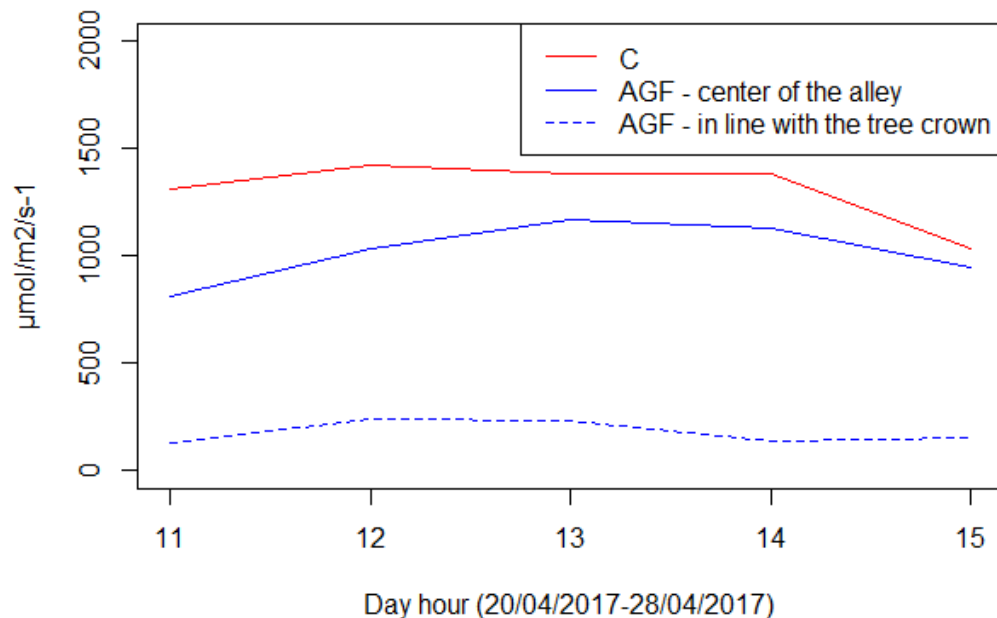


Figure 5 PAR in AGF treatment reaching two positions: the soil at the center of the alley (3 m from olive trunk) and the soil in line with the tree crown. Average of 8 days (from 20/04/2017 till 28/04/2017) of recording and for the central hours of the day. The control PAR data in the same period are also showed

PAR and distance to the trees. In AGF treatment, the difference between the PAR reaching the soil at the center of the alley (3 m from olive trunk) and the PAR reaching the soil in line with the tree crown (1 m from olives trunk) was significant. The reduction compared to full sun conditions, ranged from 32 % in the middle of the alley to 78 % under the olive tree canopy (Figure 5).

3.2 Air temperature and relative humidity

As there were no significant differences between AGF and C treatments considering daily maximum and minimum temperatures (Table 3), the diurnal temperature cycle was considered and the difference between AGF and C was computed for each hour (figure 6-left).

A “buffer effect” was then showed in the agroforestry system: air temperature was lower during day hours and higher during night hours. Specifically, the difference was negligible from midnight to 5 am (- 0.4°C in AGF compared to C). Then from 5 am till 2 pm AGF system lowered temperature by 2 °C compared to control, with a maximum of – 3,2 °C at 9 am. During night hours, from 3 pm to midnight, air temperature was higher in AGF than in C, with a maximum + 1,7°C at 7 pm.

Relative humidity was analyzed as air temperature (Figure 6-right). From 5 am to midday relative humidity was higher in AGF treatment with a maximal difference at 9 am (+8% of HR in agroforestry treatments). From midday till midnight and in the first 5 hours of the day relative humidity is higher in the control system than in AGF one. The maximal difference was at 8 pm with + 9 % of relative humidity in open field condition compared to agroforestry.

Table 3 Average maximum and minimum temperatures (°C) for system AGF and C during 3 different development stage. (BBCH scale was used to determine phenological stage). Germination- Stem elongation period considers air temperature between 20 December 2016 to 10 Mars 2017; Stem elongation – Anthesis considers air temperature between 11 Mars 2017 to 24 April; Anthesis-Maturity consider air temperature between 25 Avril to 23 June 2017 (harvest).

	Treatment	Tmax (°C)	Tmin (°C)
Germination - Stem elongation	AGF	20,3	-6,5
	C	20,6	-5,1
Stem elongation – Anthesis	AGF	25,8	2,4
	C	25,8	2,3
Anthesis- Maturity	AGF	35,7	4,2
	C	35,9	4,8

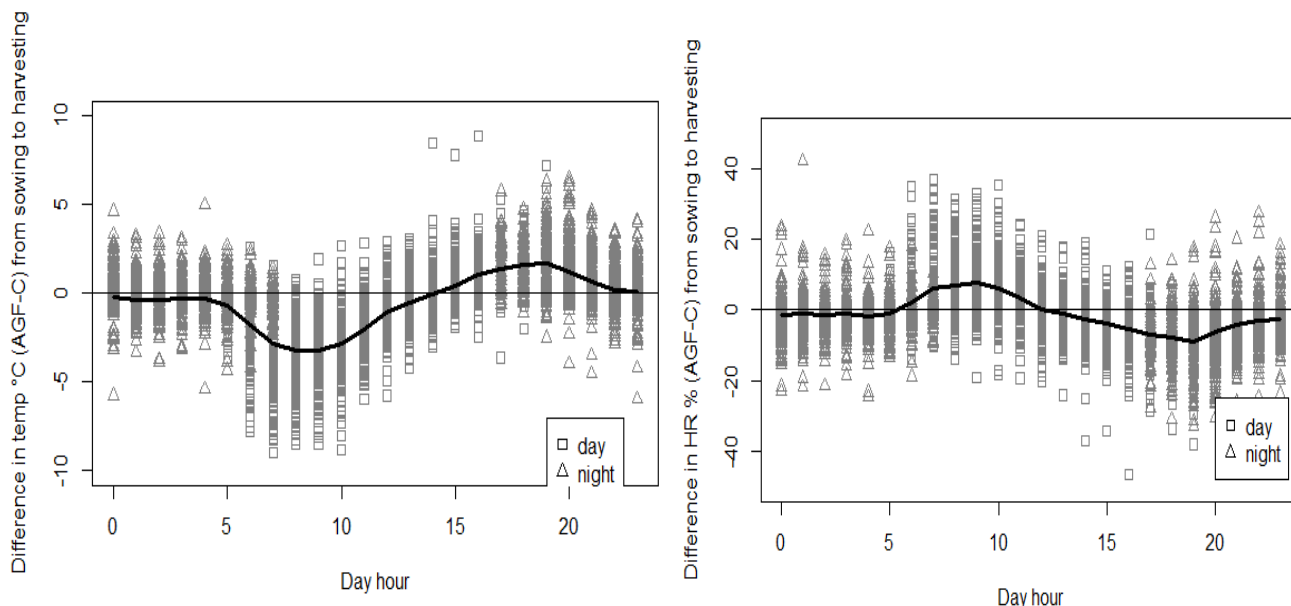


Figure 6 . left) Difference in temperature (AGF-C as °C) for each hour of the day during durum wheat cycle (December 2016-June 2017). right) Difference in air relative humidity (AGF-C as % relative humidity) for each hour of the day during durum wheat cycle (December 2016-June 2017).

3.3 Wind speed

Wind speed was significantly different between treatments, with the greatest difference during the period: January-march (figure 7). Considering the daily average wind velocity, the open field conditions (C) was characterized by 2,0 m/s, AGF by 0,25 m/s and AGF+ by 0,01 m/s. The wind speed reached maximal values of 24 m/s in the Control treatment, 9.8 m/s and 4.5 in AGF and AGF+ treatments respectively. HSD test showed significance in the difference among the 3 treatments for both parameters.

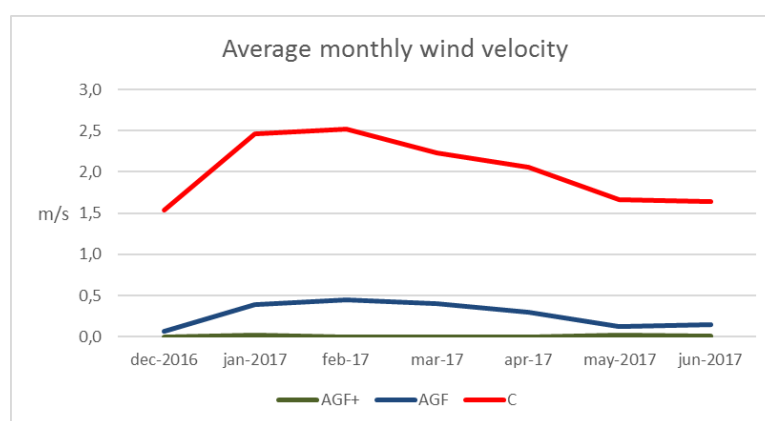


Figure 7 Average monthly wind velocity

All over the period of wind measurements (from 01/12/2016 till 26/06/2017), the number of hours with maximum speed wind >5,4 m/s (i.e 19 km/h = level 4 in the Beaufort scale) is equal to 1356 hour, equivalent to 60 days for the control treatment (Table 4). This level 4, corresponding to a moderate breeze, is a threshold for chemical treatments application. In AGF treatment the number of hours with maximum wind speed > 5,4 m/s was equivalent to 4 days, in AGF+ treatment such wind speed was never observed.

Table 4 Number of hours where means and maximal wind velocity > 5.4 m/s for the three treatments.

Wind speed	control	AGF	AGF+
Hour mean >5.4 m/s (19 Km/h)	223 hours	3 hours	0
Hour maximum > 5.4 m/s	1356 hours	89 hours	0

3.4 Edaphic environment

3.4.1 Soil moisture content

The changes in soil water content with crop growing seasons, soil depth and according to the treatments are shown in Figure 8. From December to April, soil water potential ranged between 0 and -50 KPa at any depth and in each treatment. From the end of April (phenological stage of anthesis – BBCH scale) till harvest (at end of June), a decrease in water content was recorded with different proportions according to the treatments and depths. At any depth, AGF+ was the treatment having the lower pressure potential recorded, thus its soil had the highest water content during the whole season.

The value of -300 KPa is the limit for reliable values recorded by watermark probes. Values surpassing this threshold indicate a very low water content in the soil. AGF+ reached this value only in the first 30 cm of soil and only at end of June, whereas C and AGF treatments surpassed this value at any depth and for a longer period (from the end of May till whole June at 30-60-90 cm in the soil, and just at the end of June at 110 cm in the soil).

In the first 30 cm of the soil, AGF and C showed similar water content dynamic through months. Considering the period between end of April and beginning of June, the average pressure potential was -260 KP for C and AGF and 5 times higher for AGF+ (-54 KPa). Then, from 60cm to 110 cm depth, the difference between agroforestry systems and the control decreased progressively. At 60 cm depth, from end of April till beginning of June, pressure potential was average 284 in C treatment and it increased by 4 times in AGF (73 KPa) and by 7

times in AGF+ (40 KPa). At 90 and 110 cm depth the average pressure potential in AGF was 2 times higher than in C, and 3 times in AGF+.

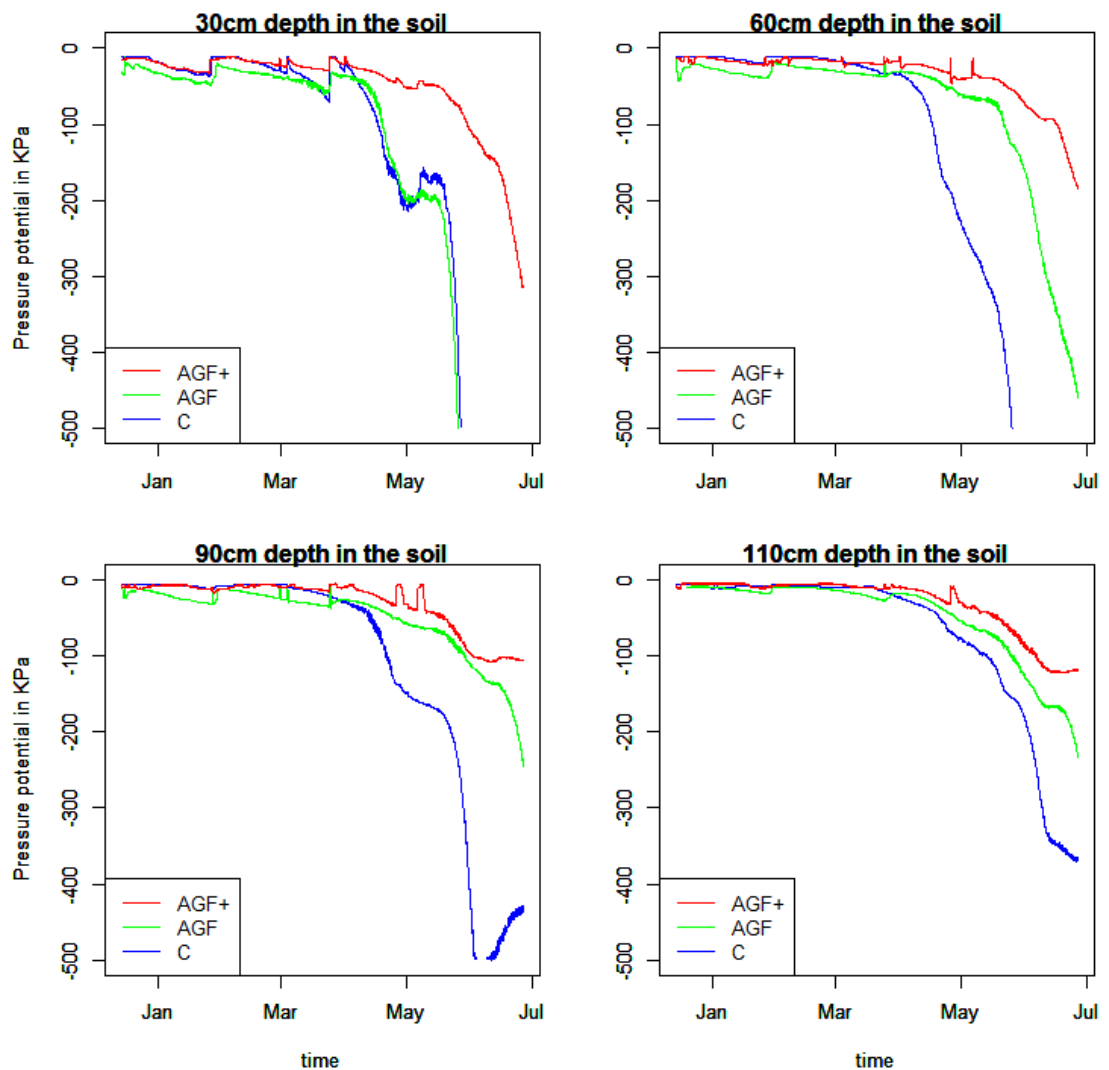


Figure 8 Soil water content at 30-60-90-110 cm of depth in the soil. Values are expressed in pressure potential: the force with which water is held in the soil. When pressure potential value is 0 KPa the soil is saturated by water; the more this value gets negative, the more the soil is in water stress conditions.

3.4.2 Chemical soil properties

Chemical soil properties were investigated through soil samples analyses in 3 soil horizons (HOR1= 0-30 cm, HOR2 =30-60 cm, HOR3= 60-90 cm). No significant difference was found between treatments, except for NO_3^- concentration in HOR3, which was significantly higher in C treatment (Table 5). Considering NH_4^+ concentration, although no statistical difference was noted due to variability, a tendency was observed in the 3 horizons: AGF+ had slightly higher content with average 2.11 mg/Kg TS, AGF showed a NH_4^+ concentration decreased on average

by 37%, then C showed the lowest concentration decreased by average 54 % compared to AGF+.

NO₃⁻ showed higher concentrations in agroforestry treatment in the first 30 cm of soil profile (AGF +34 % compared to C). In the two deepest soil horizons, the highest NO₃⁻ concentrations were in C system (+ 8% in Hor2 compared to AGF, +50% in Hor3 compared to AGF).

Table 5 NH₄⁺ and NO₃⁻ concentration in the soil (mg/Kg TS, where TS=total solids) from soil samples from the 3 systems, extracted and analysed in February 2017. Hor1 corresponds to 0-30 cm depth in the soil, Hor2=30-60 cm, Hor3=60-90 cm. Means with different letters are significantly different according to Tukey's HSD

	HOR 1		HOR 2		HOR 3	
	NH ₄ ⁺	NO ₃ ⁻	NH ₄ ⁺	NO ₃ ⁻	NH ₄ ⁺	NO ₃ ⁻
AGF+	3,07 (±0,8) a	2,8 (±1,9) a	1,78 (±0,9) a	1,01 (±0,5) ab	1,49 (±0,5) a	0,78 (±0,6) c
AGF	1,5 (±0,8) a	3,2 (±1,2) a	1,22 (±0,5) a	2,09 (±1,8) a	1,24 (±0,6) a	1,78 (±1,3) b
C	0,92 (±0,5) a	2,1 (±0,5) a	1,08 (±0,2) a	2,28 (±1,3) a	0,94 (±0,4) a	3,5 (±1,6) a

3.4.3 Mycorrhizal colonization

Agroforestry systems is often presented as perennial systems that promotes beneficial biological interactions between microorganisms and plant species, especially those formed by arbuscular mycorrhizal fungi (AMF) and roots. Our aim was to verify this assertion by comparing the colonization rate of AMF in wheat roots between the 3 treatments. Four parameters were considered: (i) the frequency and intensity of mycorrhizal colonization in the root system, (ii) the intensity of the mycorrhizal colonization in the root fragments, (iii) the arbuscular abundance in mycorrhizal parts of root fragments and (iv) the arbuscular abundance in the root system.

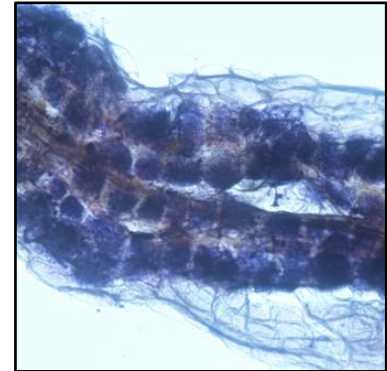
Table 6 shows the mean values obtained after the analyses of the roots of 6 durum wheat pure lines: 1, 4, 101, 43, 45, 266. A number of 270 root fragments of 1 cm in length were observed for each variety and each treatment. The % of fragment colonized by AMF was generally very high within all treatments.

The totality (100%) of root fragments observed from AGF samples were colonized compared to the 96.4% of the control. The intensity of the colonization, which express the % of the root fragment length to be colonized, was significantly higher in agroforestry treatments with best

results for AGF (+34% compared to C). Considering the abundance of AMF arbuscular in the root systems, it was significantly higher in the two agroforestry treatments.

Table 6 *Michorriza* colonization results through 3 main parameters: Frequency of mycorrhization in the root system (% of colonized fragments), Intensity of the mycorrhizal colonization (% of fragment length colonized) and Arbuscular Abundance in the root system. Means with different letters are significantly different according to Tukey's HSD. On the right a photo of arbuscules in AGF durum wheat roots.

	Frequency %	Intensity %	Arbuscular abundance %
AGF	100 (±0) a	66 (±8,5) a	30 (±4) a
AGF+	99,8 (±0,8) a	61,7 (±14,4) a	28,6 (±9) a
C	96,4 (6,7) b	43,7 (18,4) b	17,2 (10,4) b



4. Discussion

Impact of tree pruning, tree row orientation and spacing on PAR. In this experiment the two agroforestry treatments showed a reduction in PAR reaching the crop, by 33 % for AGF and 55% for AGF+, respectively, compared to the controls C. Several authors assessed an increasing yield reduction with increasing shade levels in wheat as wheat as well as in other crops (Li et al. 2008, Dufour et al. 2013, Gillespie et al. 2000). Competition for light is then considered as main issue when assessing the potential of an agroforestry system. Li et al (2008) highlighted that tree shape and pruning, tree row orientation and spacing and also tree phenology can reduce the effect of the tree shade on the crop. For instance, by intercropping durum wheat with Pawlonia tree, these authors observed reduction in incoming PAR of 22%, 44% and 56% during flowering, grain filling and maturity. In the south of France, in a site 20 Km Northern of our site experiment, an agroforestry system including walnut trees and durum wheat was tested (Dufour et al. 2013). With 31 % of light reduction which is comparable to our AGF treatment (33 % of PAR reduction), yield was decreased by 50 %. In this case, the combination of a winter cereal and a late deciduous tree showed to be favourable as walnut budburst occurred when LAI max stage of crop was past. In our case, olive trees producing evergreen leaves and compared to deciduous tree species, it leads to shade effects on the understored crop for a longer period of crop growth. The orientation of tree rows was also seen to be relevant. Some authors stated that north-south orientations are less competitive for light than east-west rows (Dufour et al. 2013, Gillespie et al. 2000). Our olive orchard, oriented

north-west south-east, may lead to a higher shade impact compared to a possible north-south orientation. However, further studies in order to assess this phenomenon are required.

In our experiment, the distance between the olive tree rows is very short (6m) compared to classical agroforestry designs (from 15 to 25 m). This short distance leads to a higher PAR reduction for the plants located between tree rows and for part of them directly under the foliage crown. Yang et al. (2016) studied the spatial variation in maize PAR based on distance from rows of trees and height positions of maize. During the ear forming growth of maize, the high density of the jujube tree leaves significantly reduced the PAR of maize plants, especially those growing closer to the tree row (2.5 m and 3.5 m) and in the morning (before 11:00). During midday (11:00–15:00), the maize plants received more PAR at 3.5 m than at 2.5 m or 5.5 m from tree row. For each maize plant, the higher the canopy, the more PAR it received.

Windbreak role and impact on wheat. Olive trees slow the movement of air thus reducing both average (from 87% with pruned trees to 99% with unpruned trees) and maximum (from 60% to 85% respectively) wind speed. For farmers, wind velocity is important to know for instance to plan chemical treatment days. Indeed, in France, crop treatments are forbidden when wind speed is superior to a threshold of 19 km/h (i.e., 5,2 m/s: level 4 in Beaufort scale). The maximum value for wind speed reached in Control treatment was 24 m/s corresponding to level 9 on Beaufort Scale. It is considered as “Strong/severe gale”. This level was observed during 4 hours. The presence of trees avoids to reach this level, and the maximum obtained for AGF corresponds to level 5 (Fresh breeze), and to level 3 (Gentle breeze) in AGF+ treatment.

Although it is largely spread that Agroforestry, thanks to its windbreak effect, has a positive impact on temperature, relative humidity of the air and infiltration of rain water, some authors (Auclair et Dupraz, 2013) didn't observed such impact.

Concerning temperature. Agroforestry is claimed between strategical practices to protect heat sensitive crops (Rao et al. 2007) and generally to face climate change prevision. Beer et al (1998), studying coffee and cacao plantations, have observed that shade trees buffer high and low temperature extremes by as much as 5 °C. In our study, we did not find significantly difference in maximal and minimal temperature between agroforestry and full sun conditions. The buffer effect was observed between daytime temperature and night time temperature: durum wheat in the agroforestry system experienced lower temperature during the day (max 3.2°C) and higher temperature during the night (max. 1.7 °C) compared to full sun conditions. Lin et al (2007) found the same results within a coffee agroforestry systems in Southern Mexico and both for dry and wet season. Recently, the same pattern was observed in durum wheat-walnut

agroforestry system in south of France (Inurreta et al. 2017, Gosme et al. 2016): the mean difference was $-1.2\text{ }^{\circ}\text{C}$ on clear days vs. $-0.27\text{ }^{\circ}\text{C}$ on cloudy days (respectively 1.17 during the day vs. 0.43 of night). Reducing daily temperature may have beneficial effects on crop (Rao et al. 2007) face to prevision of warming conditions. Warm night, on the contrary, could have negative impact on crop growth and consequently yield. Garcia et al, 2016, using purpose-built heating chambers, exposed durum wheat and barley to ambient and high night temperatures: yield was reduced by 7% for each temperature degree of increase during night time. To explain this process, accelerated development rate and lower C assimilation rate due to higher dark respiration were proposed by Grant et al., 2011.

Concerning relative humidity. Relative humidity (RH) generally follows the rainfall pattern. Relative humidity showed also a day-night cycle. Although a higher relative humidity level is cited among the numerous effects of agroforestry systems, this phenomenon was noticed only during some hours of the day. In our study, RH was higher between olive trees only from 5 am to midday (max +9 %) compared to open field conditions, and lower for the rest of the day. Lin et al 2007 obtained similar results but for a longer period of the day as higher relative humidity was recorded from 8 am to 16 pm in a coffee agroforestry system. According to these authors, lower humidity during night hours may not affect plant negatively as daytime measurements are more important in determining water use in the plants.

Windbreaks are also known to reduce evaporative water losses and then to conserve soil moisture (Rao et al. 2007, Cleugh et al. 1998, Jose et al. 2004).

Considering water balance. In our study, water content in the soil increased as shade level increased. In particular, the system with higher shade level (55% of light reduction) had 5 times higher soil moisture compared to the Control in the first 30 cm of soil, 7 times more at 60 cm depth and then 2 times more till 110 cm depth in the soil. The medium shade system (32% of PAR reduction), then, showed the same water content than the full sun condition in the first soil layer (0-30 cm); in the deeper layers it had averagely 3 times higher soil moisture content compared to the Control. Lin et al 2009, studying an agroforestry system with 30 % of shade cover, similarly to AGF in our experiment, found a significant reduction of 32 % in evaporative transpiration demand. Some authors are also considering the process of hydraulic lift as possible explication (Lin et al. 2009, Jose et al. 2004, Smith et al. 2013, Schoeneberger et al. 2012, Ong et al. 1999): some deep-rooted plants would be able to take water from lower soil layers and release it into upper drier soil layers. Although this process has been reported in species as *Quercus*, and *Pinus* with potential for agroforestry (Filella 2003), direct beneficial evidence is not yet available from temperate agroforestry systems (Jose et al. 2004).

But literature provides contrasting results within different agroforestry systems. Some authors stated that water is the main limiting factor when intercropping woody plants and crops. Gillespie et al. (2000), while intercropping maize with oak, observed no yield reduction when belowground competitions for water and nutrients was eliminated through polyethylene barriers. Wanvestraut et al. (2004) confirmed this result with a 26% of cotton yield reduction in the no barrier treatment. Even if it is difficult to separate the belowground competition for water from that for nutrients, there is a part of the literature which affirms that crop production in agroforestry systems in semiarid regions is limited mainly by competition for water (Jose et al. 2004, Leihner et al. 1996, Gillespie et al. 2000, Lin et al. 1999, Pallardy et al. 2001).

On the other hand, several authors found agroforestry systems to reduce water losses by reducing the amount of water lost through soil evaporation and crop transpiration. Lin et al 2009, for instance, studied a high (60-80%), medium (35-65%) and a low (10-30%) shade coffee agroforestry system and observed that the low shade system presents the lowest soil moisture level. They conclude that in full sun condition the crop experience a higher transpiration demand and they highlighted the role of trees as windbreak. Wind energy, reduced within agroforestry systems, carry water away from soil and leaf surfaces, thus increasing evapotranspiration demand. There are several mechanisms through which agroforestry may use available water more efficiently than annual crop (Rao et al. 2007): perennial trees use water remaining in the soil after harvesting, they capture a larger proportion of rainfall by reducing the runoff and by using water in the deeper soil. Additionally, the change in the microclimate (temperature and wind speed) reduce the evaporative demand and make more water available for transpiration (Cleugh et al. 1998, Jose et al. 2004, Smith et al. 2013).

Agroforestry and Soil fertility. In our experiment we found higher NH_4^+ level in Agroforestry treatments than in Control, and less NO_3^- . Nitrogen fixation is the process by which gaseous nitrogen (N_2) is converted to ammonia (NH_3 or NH_4^+) via biological fixation. A small group of bacteria and cyanobacteria are capable to use the enzyme nitrogenase to break the bonds among the molecular nitrogen N_2 and combine it with hydrogen. Nitrification is a two-step process in which $\text{NH}_3/\text{NH}_4^+$ is converted into NO_3^- . First, the soil bacteria *Nitrosomonas* and *Nitrococcus* convert NH_3 to NO_2^- , and then another soil bacterium, *Nitrobacter*, oxidizes NO_2^- to NO_3^- . These bacteria gain energy through these conversions, both of which require oxygen to occur. Our agroforestry system had higher levels of microbial biomass and potentially mineralizable N, but lower levels of NO_3^- . With regard to the soil functioning to protect the environment by decreasing the potential for $\text{NO}_3\text{-N}$ leaching, this was interpreted to indicate an improved soil quality (Karlen et al. 1997). However, the trade-off was that the higher residue cover and lower

potential N leaching losses during the non-growing season resulted in lower available N, which was a potential limitation with regard to the soil functioning for wheat production.

5. Conclusions

The presence of olive trees modifies the microclimate experienced by the understorey crops compared to crops grown in pure agricultural fields. Trees shade the crop and reduce wind velocity, thus altering temperature and water balance in the alley. Olive tree rows by creating barriers to wind and reducing radiation reaching the crop, modify temperature and water availability in the alley. The edaphic environment of agroforestry treatments seems then to be favourable for the proliferation of these arbuscular mycorrhizal fungi within crop roots.

II. EFFECT OF AGROFORESTRY ON PHENOLOGY OF DIFFERENT VARIETIES OF DURUM WHEAT

1. Introduction

In the Mediterranean region, durum wheat productivity is mainly affected by heat stress and summer drought. This situation is expected to intensify in the future (IFPRI), as average temperature and extreme events frequency will increase (Moriondo et al. 2007). Several climate models are nowadays available to create probabilistic projection of climate change impact on durum wheat yield (Ferrise et al. 2011). Provisions of yield reduction run up to 20-30% in developing countries accordingly to several authors (Esterling et al., 2007; Rosegrant and Agcaoili 2010).

Crop phenology is one important plant parameter in determining final yield. The effect of warming climate on crop phenology has been indicated as a key-point for assessing the impact of climate change on agricultural crops (Moriondo et al. 2007). Plant phenology is mainly driven by air temperature and under heat stress the wheat crop completes its life cycle much faster; as consequence, crop growth stages will have shorter duration. This process may lead to a lower accumulation of dry matter and, hence, to lower grain yield (Al-Karaki et al. 2012; Ferrise et al. 2011, Carboni et al. 2011).

Agroforestry modifies environmental conditions for the understorey crop. In the first study (article I, paragraph 3.1) we observed that, while determining a reduction in solar radiation reaching the crop (-33% PAR in AGF treatment, -55% PAR in AGF+), trees modifies the diurnal cycle temperature, the relative humidity and the water retention in the inter-row. A buffer effect was seen in daily temperature as agroforestry increased temperature during night hour (+ 1.7°C max) and decreased it during a part of day hours (-2°C on the average). Relative humidity was also altered with higher values during the day and lower during night, while wind speed was reduced. The question is: does the modification of environment lead to a modification of the phenology of the plant?

In this article, we studied the impact of olive trees on the phenology of 25 durum-wheat varieties intercropped in the Mediterranean context of southern France.

2. Material and methods

The **Experimental design** was described in article I, paragraph 2.2.

2.1 Data collection

Microclimate parameters were recorded from wheat sowing in winter to wheat harvesting in the end of June. A permanent Meteo-France weather station and tools placed in each one of the 3 treatment monitored microclimate as described in 2 of second section.

The BBCH (BASF, Bayer, Ciba-Geigy and Hoechst) scale was used to describe the growth stage of wheat (Lancashire et al., 1991; Zadoks et al., 1974), as shown in Figure 9. Wheat phenology was recorded weekly from the beginning of stage 20 "tillering" (February 22) to the beginning of stage 70 "development of kernel" (May 15). As wheat populations varieties showed an heterogeneity in phenological stage among the different plants in the plot for the same date of recording; it was decided to note the most advanced growth stage showed by plants of each population.

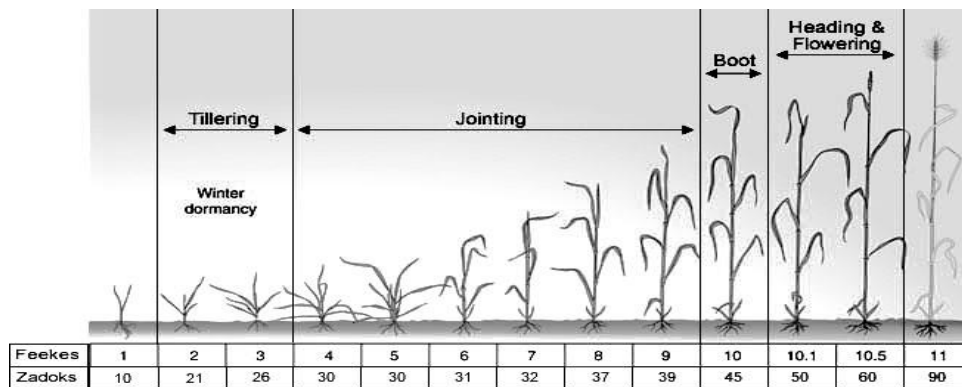


Figure 9. The BBCH (BASF, Bayer, Ciba-Geigy and Hoechst) scale was used to describe the growth stage of wheat (Lancashire et al., 1991; Zadoks et al., 1974)

2.2 Data analysis

Firstly, durum wheat phenology was analysed by observing the difference between treatment and the difference between genotypes was consider as environmental variability inside each treatment. Secondly, phenology was deeply investigated by observing the phenological development of 10 genotype among the 25, within each treatment. The 10 genotypes were chosen in order to represent the genetical variability of the all genotypes: genotypes 1, 2, 4, 100 and 101 are modern pure lines, genotypes 43 and 45 are "ancient" pure lines varieties taken out of the genebank maintained by INRA, genotypes 14, 56 and 59 are populations.

3. Results

In a first moment, the phenological development of durum wheat in the three treatments was analysed by considering different genotypes as random effect of the environment. Figure 10 shows the average phenological stage reached by the 25 genotypes within each treatment for each date of notation.

We can observe 3 periods. At the end of winter, between the end of February and the beginning of March, the treatments were at significantly different growing stages: at the first date of notations (22/02/2017), while AGF+ was still unfolding his leaves (BBCH=15= 5 leaves unfolded), AGF and then C were already developing the tillers (AGF at BBCH=20, C with already 2 tillers detectable). This ranking among treatments, with AGF+ with the slowest phenological growing and C treatment with the fastest, lasted till the second week of March. Then we can observe the second period: from the second week of March till the end of the month, BBCH stages noted didn't show differences among treatments. All of them were at the phenological stage of "stem elongation" (BBCH=30). The BBCH scale defines the progression of the phenological stage, i.e., 30, 31, 32, $3n$ with n being the number of detectable nodes. As we could observe 1, 2 or max. 3 nodes (BBCH=30, 31, 32) in the main stem of plant, during the stage "stem elongation" no significant differences were seen among treatments.

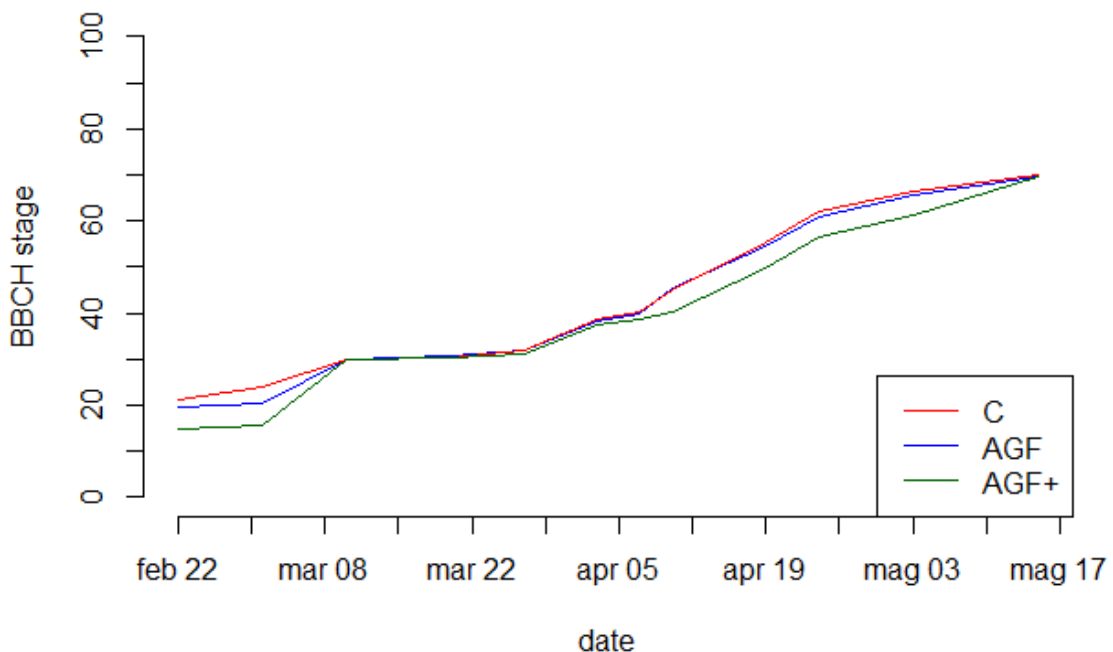


Figure 10 Phenological development of durum wheat in the three treatments (average of 25 varieties). Date of phenology notations on x axis, BBCH stage noted in y axis.

Then, from the beginning of booting (BBCH=40), we can distinguish the third period, which showed the same tendency as the first period. From the second week of April till the last date of notation, the control treatment showed the faster phenological development, then AGF was intermediate between the other two and AGF+ was the slower to reach each phenological stage. Moreover, what we can observe, is that the distance between AGF+ and C was greater than the one between AGF and C.

But these average data are hiding the variability among different varieties. In a second moment, we observed the phenological development of 10 genotypes in the three treatments. Table 7 shows the number of days after sowing (DAS) that each variety in the control treatment took to reach two main phenological stages considered: BBCH= 47 which define the “flag leaf sheath opening” stage (Zadoks et al., 1974), that precedes the beginning of heading, and BBCH=69 which define the end of the anthesis. Then, AGF and AGF+ are described with the difference in number of days compared to the control to attain the two same stages.

The 10 genotypes grown in the Control treatment reached BBCH=47 averagely 134 days after sowing (15 April). But a variability is shown: some genotypes (var. 4, 14, 56, 100) had an earlier development, reaching BBCH=47 5 to 8 days before the average. Others (var. 59 and 45) showed a later development with 7 to 17 days of retard compared to the average. AGF treatment attained the pre-heading (17 April) 2 days on average after the control with a variability ranging from 0 to 5 (var. 59) between AGF and C. Plants in AGF+ treatment reached the pre-heading (21 April) 6 days after the Control on average, with some genotypes showing more retard compared to others (var 45 with a difference of 14 days compared to the Control and var 59 and 101 with 10 days of retard).

The end of anthesis was attained averagely 147 (28 Avril) days after sowing for the 10 genotypes grown in the control treatment. This phenological stage was reached averagely 4 days later by AGF (02 May) treatment and 9 days later by AGF+ treatment (07 May). A variability was observed: from 0 to +10 (var. 43) days for AGF and from +1 to +14 (var 100) for AGF+.

Table 7 10 Genotypes considered among the 25. The number of days after sowing (DAS) that each variety in the control treatment took to reach two main phenological stages considered: BBCH= 47 (flag leaf sheath opening) and BBCH=69 (end of anthesis). Then, AGF and AGF+ are described with the difference in number of days compared to the control to attain the two same stages

GENOTYPE	PRE-HEADING - BBCH=47			END OF ANTHESIS - BBCH=69		
	number of days after sowing	Difference of days compared to control	Difference of days compared to control	number of days after sowing	Difference of days compared to control	Difference of days compared to control
	C	AGF	AGF+	C	AGF	AGF+
1	138	0	+3	148	+3	+13
2	138	+2	+2	152	+3	+7
4	125	+1	+6	138	+2	+5
14	127	0	+7	143	0	+1
43	130	0	+4	141	+10	+13
45	150	+4	+14	159	+8	+12
56	127	+1	+2	139	+3	+6
59	140	+5	+10	156	+3	+8
100	128	0	+6	141	+3	+14
101	133	+4	+10	150	+3	+10
MEAN	133.6	+1.7	+6.4	146.7	+3.8	+8.9

Among the 25 genotypes, the phenological development of the varieties showed a different tendency compared the one of populations from BBCH=40. In AGF+ treatment no significant differences were observed between the two genotype types for any date of notation. In the Control and the AGF treatment the populations genotypes reached each phenological stage after BBCH=40 faster than the wheat varieties. The difference between the two types was significant in three dates (19/04 – 24/04 – 03/05) for the AGF treatment, and in two dates (19/04 – 24/04) for the Control Treatment.

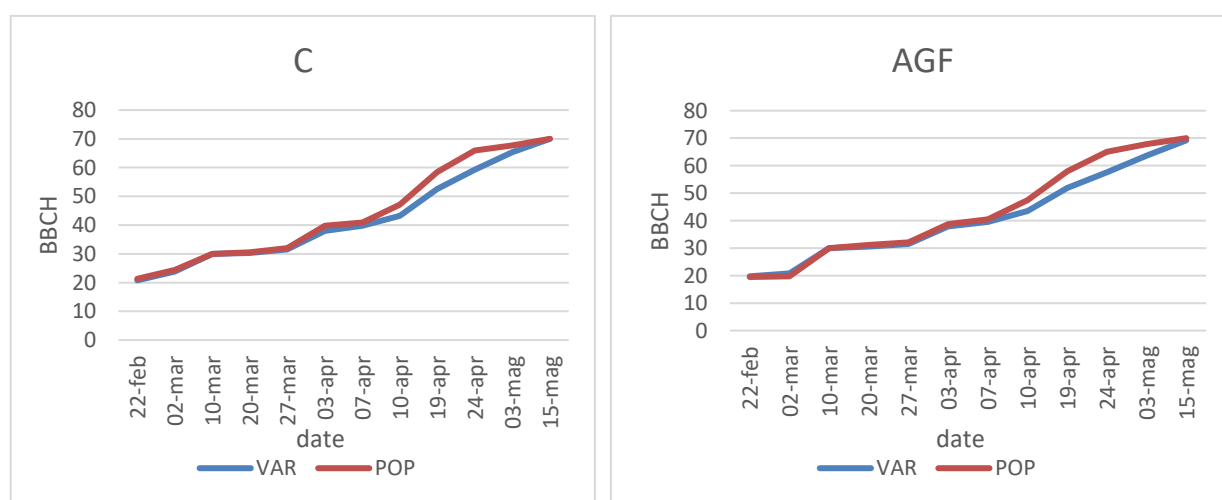


Figure 11 Phenological development of durum wheat varieties and populations for Control treatment and AGF treatment. Date of phenology notations on x axis, BBCH stage noted in y axis.

4. Discussion

Plants phenology is one of the most important plant process in determining final yield and adaptation of crops to climate change. Facing to the increase in mean temperature predicted for the near future, there is the need to find adaptation strategies which assure crop productivity. Durum wheat phenology in response to thermal stress has been widely investigated (Tubiello et al. 2000; Hossain et al. 2012; Moriondo et al. 2007; Ferrise et al. 2011), as the productivity of this crop assure essential food worldwide.

As observed in these studies, higher temperatures, which increase the crop development rate, shorten the crop growing cycle, generally reduce the time for biomass accumulation, and consequently final yield. Our hypothesis was that agroforestry farming, modifying the microclimate to which the crop under stored is exposed, might slow down crop phenological development and thus reduce the negative impact of climate change. In our study, we observed that durum wheat grown under agroforestry condition, slowed its phenology compared to full sun conditions: from 2 to 9 days of difference were noted according to the stage considered and the variety observed. This retard showed to increase with increased shade level as to attain the 2 phenological stage considered AGF+ (-55% PAR) treatment took average 8 days more than the control and AGF (-33% PAR) on average 3 days more. The magnitude of this slow in phenology within agroforestry treatment, moreover, showed a high variability among genotypes (from 0 to 14 days of difference).

Recent studies showed that the variation in length of the pre-heading stage influences grain yield more than variation in length of grain filling (Al-Karaki et al. 2012). Among the 10 genotypes we investigated the pre-heading stage was extended by 5 days at max. (var. 59) in AGF treatment and by 14 days as maximum (var. 45) in AGF+ treatment.

5. Conclusions

From these first results, agroforestry systems seem to determine several modifications of the environmental conditions which has an impact on crop phenology, variable within phenological stages and varieties. To deeply understand the potential of these impact on phenology, it will be interesting to focus on the durum wheat genotypes showing the most important slowing in phenology, and see what was the impact on yield and yield components.

EFFECT OF AGROFORESTRY ON YIELD, YIELD COMPONENTS AND MORPHOLOGY OF DIFFERENT VARIETIES OF DURUM WHEAT

1. Introduction

Agroforestry is mainly defined as an approach to land use that incorporate trees into farming systems (Chahan et al. 2010) but our purpose is, on the contrary, to introduce crops into tree systems and therefore to identify the best varieties suitable for agroforestry. What should be a durum wheat ideotype well adapted to grow up in olive trees orchard? What should be the main breeding criteria? Are they different to those sought in conventional breeding?

Yield remains the critical issue even for sustainable agroforestry systems. While growing durum wheat crop under trees, light competition has been defined as the main limiting resource determining crop yield reduction (Dufour et al. 2013). Recent studies investigated the impact of different light regime experienced by durum wheat under temperate agroforestry systems. There is a variability in the impact on yield and yield components found within experimental results available today. Total yield reduction while intercropping durum wheat with trees has been assessed from 20 to 50 % compared to open field conditions (Artru et al. 2017; Dufour et al. 2013; Li et al. 2008). Artru et al (2017) showed that a reduction of 61% and 43% of the global radiation induced a final yield reduction of 45% and 25% respectively. Therefore, the correlation between grain yield and shade intensity was not linear.

Yield components are useful to understand wheat response to different levels and period of shade application. While the number of spike per square meter was not affected by agroforestry conditions (Dufour et al. 2013; Wang et al. 2014), spike biomass was significantly reduced (Artru et al 2017). According to Dufour et al. (2013), the main effect of the shade was the reduction in the number of grains per spike (35% at the most). Other studies found a lower impact, with average 25% of reduction (Artru et al. 2017). The kernel weight is the yield component with the higher variability according to these authors, from a moderate reduction of 10% till 32%. Concerning protein content, authors agree that the concentration in the grain increase with increasing shade (Artru et al. 2017; Dufour et al. 2013).

Additionally, as wheat varieties currently grown were selected in full light conditions, they do not have shade - tolerant traits. Several authors assume light as critical limiting factor and agree with the cruciality of developing breeding programs in order to select shade tolerant cultivars (Retkute et al. 2015; Ehret et al. 2015; Barro et al. 2012). Moreover, recent studies proposed

warmer temperature during night as key factor to explain yield impact in agroforestry conditions (Gosme et al. 2016, Garcia et al. 2015). Then, there is a need to identify which are the desirable traits of the crop for agroforestry systems.

The aim of the present work was to assess the performance of different durum wheat genotypes under temperate agroforestry conditions. The impact on yield and yield components was investigated over a variability of durum wheat genetic profiles, from pure line modern varieties till ancient populations. The experiment was carried on in an organic agroforestry system of South of France, where durum wheat was intercropped with olive trees. The experiment aimed to assess the impact of our Agroforestry model on the yield, the yield components and the morphology of the durum wheat understored.

2. Material and methods

2.1 Experimental design

The experimental design was detailed in the first section (paragraph I.2.2.). Hereafter the specificities of this experimentation are detailed.

2.2 Inputs

Sowing was directly accomplished by harrowing only the first 10 cm (without ploughing). No treatments have been done during the four-year period of the project (from 2014 till 2017), neither protection neither fertilization products; additionally, no nitrogen applications have been realized, thus defining a zero input organic system. Nitrogen supply is only driven by legumes crops (chickpea, fababean, forage mix) implemented in the system in an annual rotation with durum wheat.

2.3 Durum wheat varieties.

Table 1 is again presented as remind with the list of cultivars.

variety code	variety name	type	pure line characteristics
1	LA1823	VAR	modern
2	Clovis	VAR	modern
4	2007D023.655	VAR	modern
6	2007D003.109	VAR	modern
9	2007D010.255	VAR	modern
11	Pop Algérie 1	POP	

12	Pop Algérie 2	POP	
13	Pop Algérie 3	POP	
14	Pop F2 + lég Salernes	POP	
16	Pop F2 + lég Ampus	POP	
22	Pop F3 + lég Mauguio	POP	
43	RG 425	VAR	ancien
45	RG 137	VAR	ancien
55	Pop_PMG	POP	
56	Pop_PROT	POP	
57	Pop_Sécheresse	POP	
58	Pop_HR	POP	
59	pop NirS	POP	
77	El_Khroub_06	VAR	ancien
79	El_Khroub_10	VAR	ancien
100	Claudio	VAR	moderne
101	Dakter	VAR	moderne
102	Surmesur	VAR	moderne
266	RG 266	VAR	ancien
534	RG 534	VAR	ancien

2.4 Plant Phenotyping during the growing period

LAI - SPAD - coverage rate-growth habit. Each year and for each treatment the leaf area index, the leaf chlorophyll content and the coverage rate of each variety were recorded. LAI and chlorophyll content were recorded weekly from the last week of March till the last week of May; coverage rate was recorded twice during crop development: at BBCH stage 22 (2 tillers detectable) and at BBCH stage 41 (booting). Leaf area index was measured by a LAI-2200C (Li-cor Plant Canopy Analyzer); measures were operated within 2 durum wheat rows in the central part of the plot: 2 recordings over the canopy and 5 recordings below the canopy between the 2 durum wheat rows (3 next to wheat stems of the rows, 2 in the middle of the inter-row). 90° view restricting cap was used. The time of the day when LAI was recorded was noted. Data were collected by keeping the optical sensor to north direction for AGF and AGF+ plots (wheat rows sowed in north-south direction) and to east direction for C plots (wheat rows sown in west-east direction).

Chlorophyll content data were collected with a SPAD-502 chlorophyll meter. 6 wheat plants randomly chosen for each plot were tagged and SPAD recordings were done every week on these plants (on the last developed one?). The coverage rate was determined on 07/04/2017 at BBCH=39 (flag leaf fully unrolled, ligule just visible) using a 1-5 visual scale, where 1=0% of visible ground soil between rows and 5= >80% of visible ground soil between rows. Coverage rate was determined by observing the 2 central wheat rows of each plot. Growth habit was

determined on 27/03/2017 at the end of tillering (BBCH phenological stage) using a 1-9 visual scale regarding the leaves (the most developed ones) where 1=erect, 3=half-erect, 5=half erect-half prostrate, 7=half prostrate, 9=prostrate. The height of plants (vegetative cover) was measured weekly from the end of tillering till maturity by 3 measure/block/variety/treatment.

2.5. Plant phenotyping at maturity

Samples of plants for each treatment/variety/repetition: the last week of June 2017, 3 days before the mechanized harvest, all the plants present in a rectangular area defined by the width of 2 wheat rows and the length of 40 cm, were submitted to various measures: number of established plants, number of tillers and number of spikes were counted. Plants were then cut and spikes separated from straw. Spike and straw were dried in an oven at 80 °C for 48 h to determine the dry weight of each. Then spikes were thrashed and grains were weighed and counted. For each sample the kernel weight and the harvest index were calculated. Furthermore, 6 plants of each sample were used to determine the following measures: plant height (from coleoptile to the end of awns), spike length (without awns), spike and awn length, distance from flag leaf and the spike. Yield components were then calculated on square meter base.

2.6 Mechanical harvest

Mechanical harvest was done from 27 to 30 June 2017. Grains of each plot were collected in coded bags. Each bag was weighed directly after harvesting to measure the gross weight. Humidity of gross yield was determined. Straws were eliminated but broken and small grains were kept and collected for each sample. Net weight was recorded and humidity rate of cleaned grains was measured. For each variety/repetition three sample of 500 grains were weighed and kernel weight was then determined.

2.7 Quality analysis

2.7.1 Unvitreous measurements

The rate of loss of vitreous aspect of each durum wheat variety (“mitadinage”) was determined for each variety in each treatment and each repetition. 100 grains were observed and a visual estimation was assessed through the following 0-2 scoring: 0 = grain entirely vitreous, 100% vitreous aspect; 1= 1-2 spots on unvitreous grain surface; 3 = grain with more than 50 % of the surface that is unvitreous. This test was repeated 2 times per sample.

2.7.2 Protein content

20 grams of grains for each treatment/variety/block were milled to whole flour using a rotor mill (Cyclotec, Foss Tecator-1093, Hoganas Sweden). Protein extraction, fractionation and quantification were carried on at INRA UMR-IATE laboratory (“Ingénieries des Agropolymères et Technologies Émergentes”) in Montpellier. Protein extraction was carried on according to Dachkevitch and Autran (1989) with some modifications described in the MOP-ANA-33 protocol. 160 mg of whole flour samples were stirred for 120 min at 60°C in the presence of 20 mL of a 0.1 M sodium phosphate buffer (pH 6.9) containing 1% (p/v) sodium dodecyl sulfate (SDS), and were then centrifuged for 30 min at 18000 rpm at 20 °C to obtain a supernatant (SDS-extractable protein fraction). The Pellets were then stirred for 5 min at room temperature with 5 mL of the same extractant, and the resulting dispersion was sonicated for 3 min at 7.5 W (Morel et al., 2000), using a 3-mm diameter probe mounted on a XL—Microson ultrasonic cell disruptor (Vibra Cell 72434, Bioblock Scientific, Illkirch, France). After centrifugation (30 min, 18000 rpm, 20 °C), the supernatants (SDS-unextractable protein fractions) were collected. Proteins in the SDS-extractable and -unextractable fractions were fractionated by size-exclusion high performance liquid chromatography (SE-HPLC) using a TSKgel G 4000 SWXL column (7.8 mm I.D. x 30 cm, Tosoh Biosep, Sigma Aldrich, France) according to Dachkevitch and Autran (1989). Water content of whole flour samples was determined according to MOP-ANA-52 protocol by weighing the mass lost by 5 g of flour for each sample after being 2 h in an electric oven at 130-133°C. Protein content of each sample was then calculated over %/dry matter. The chromatography profile obtained was then divided into 5 peaks: the proportion of each protein class is deduced from the correspondent area below each peak as shown in Figure 12.

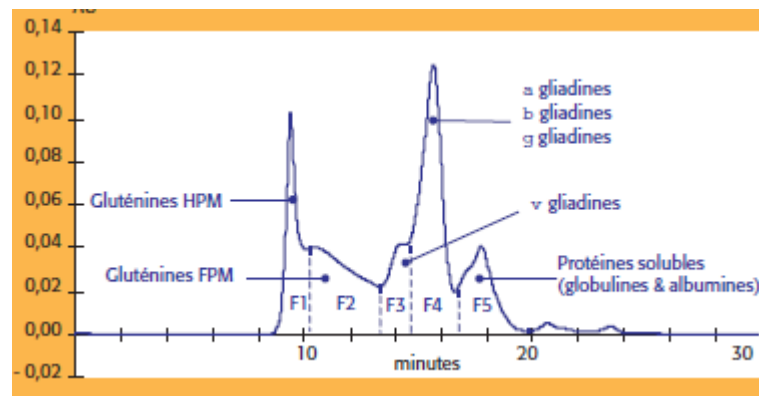


Figure 12 Chromatography profile with the five peaks obtained. Each peak corresponds to the separation of a certain protein type

3. Results and discussion

3.1 Yield and yield components

The differences in yield and yield components between systems were analysed considering different genotypes as random effect of the environment. Yield results are presented in Table 8: yield components (plants/square meter * spikes/plant * grains/spike * TGW) arise from plant phenotyping at maturity (paragraph II.2.5.) over a sampled area for each variety/block (rectangular area defined by the width of 2 wheat rows and the length of 40 cm). Considering that these sampled areas were chosen where plants showed good performances (parts flooded during winter in 4 plots were not chosen as sampled areas), yield arising only from them could be an overestimation. For this reason, to calculate Yield (quintal/ha) presented in table 8, we averaged yield arising from these sampled area and yield obtained after mechanical harvesting over the total area of each variety/block/treatment.

The average yield of all durum wheat genotypes grown in full sun conditions was significantly higher than yield reached in the 2 agroforestry systems. In AGF treatment (-33% PAR), yield was decreased by 47 % compared to control and in AGF+ treatment (-55%) it was decreased by 62 % compared to C.

Several authors have shown that reducing incident light on wheat crop leads to yield repercussions and our study is no exception. Dufour et al. (2013) and Li et al. (2008) reported that an average reduction of transmitted cumulated global radiation by 17% or 34% led to an average yield depression of 20% and 51% respectively. Artru et al. (2017) demonstrated that a reduction of 61 % and 43 % of the global radiation during the shade period induced a final reduction of 45% and 25% respectively. The average 40% reduction of yield we found in this study is then not surprising according to these recent papers. Additionally, examples found in literature investigated agroforestry systems with deciduous trees, as walnut trees (Dufour et al. 2013) or Paulownia trees (Li et al. 2008). In our case study, the olive tree is an evergreen species and thus, differently from deciduous species, the shade effect of canopy lasts for all crop growing season. Despite this, the yield reduction we observed is similar to the ones observed in studies where the shade period lasted only in the second half part of crop development (spring and summer).

According to Retkute et al. (2015), therefore, the effect of global radiation reduction on final yield depends on the phenological stage during which shade is applied, as well as the duration of the period in which the incident light is reduced. Varying these two factors will have a repercussion on the different yield components. Imposing a shade treatment during the pre-

flowering period (i.e., around 30 days before-to flowering) mainly affects final yield through the number of grain per m² component because of a change of numbers of grains per spike (Mainard and Jeuffroy, 2004; Retkute et al. 2015). However, shade from flowering to maturity reduced both number of grain per m² and grain weight (Artru et al. 2017).

The main surprising result in our experiment was the very low value of plant density. The sowing density was 350 seeds/m² while at harvest less than half of this value was still in place. This might be due to inundations just after sowing which determined flooding over the crop in December 2016. The difficulties to dry out might be responsible of the low-density values observed. Among treatments, nevertheless, AGF showed significantly higher final density compared to C (-17% in control), but still over low values compared to initial sowing density. One of the hypothesis, within agroforestry farming, is the determination of more favourable soil conditions (warmer temperature, higher humidity) in the first centimetres of the soil profile which promote a good seed emergence and a higher plant density. Inurreta et al (2016) for example, within the same durum wheat agroforestry system studied by Dufour et al. (2012) observed a crop density slightly higher in the agroforestry treatment (+10 %) compared to the Control, but over the same general low densities we observed (152 plants per square meter from 350 seeds/m²).

Considering other yield components, in our study the number of spikes per plant (where one plant means one seed sown) was significantly different among the three treatments, with a decreased of 24% for AGF treatment and of 51 % for AGF+. The most affected yield component by agroforestry treatments was the number of grains per spike, decreased by 31% and 62 % compared to control in AGF and AGF+ respectively. The 1000 grain weight was decreased only by 4 % in AGF compared to C, nevertheless a significance was seen in this difference according to Tukey's HSD test.

Results shown in literature agree with what we observed as the number of grains per spike seems to be the yield component most affected for wheat in agroforestry conditions (30% of reduction in Artru et al. 2017, 35 % in Dufour et al. 2013, 36 % in Li et al. 2008). The reduction in the weight of grains found in literature are of higher proportions compared with our results in AGF treatment. Dufour et al. (2012) found this yield component decreased by 16%, Artru et al. (2017) and Li et al. (2008) found average reductions of 20% and 25 % respectively. The reduction in the number of spikes per square meter was on average 37% in our study, while previous studies found it not affected by agroforestry (Dufour et al. 2012; Wang et al. 2003).

Plants biomass was significantly different among the three treatments: it was decreased by 52 % and by 80 % in AGF and in AGF+ respectively compared to control. Despite this difference in plants biomass, the harvest index was not significantly different between AGF treatment and the control. Li et al. (2010), while testing three shade levels on durum wheat understored (with -8%, -15 % and -23% of solar radiation), found improved redistribution of storage dry matter from vegetative organs into grains. Moreover, they found that the redistribution of dry matter from the penultimate and from the lower internodes was larger than from the intermediate internodes, especially under higher shade conditions. This indicated that shading promoted remobilization of the stored dry matter in the lower internodes and is that might have determined the similar harvest index we found between Control and AGF treatments despite the great difference in the biomass (-52% for AGF).

Table 8 Yield and yield components (means \pm SD) in C, AGF and AGF+. PL_m2=number of plants per square meter, spike_pl=number of spikes per plant (where one plant means one seed sown), gr_spike=number of grains per spike, TGW=Thousand Grain Weight, HI=harvest index. Means with different letters are significantly different according to Tukey's HSD.

System	Y (quintals/ha)	pl_m ²	spike_pl	gr_spike	TGW	Biomass (g/plant)	HI
C	25,13 (\pm 6,77) a	139,4 (\pm 29,93) b	2,41 (\pm 0,87) a	20,81 (\pm 5,31) a	52,58 (\pm 4,12) a	7,68 (\pm 2,71) a	0,40 (\pm 0,07) a
AGF	13,43 (\pm 6,33) b	168,8 (\pm 62) a	1,84 (\pm 0,57) b	14,28 (\pm 6,47) b	50,23 (\pm 3,87) b	3,69 (\pm 1,71) b	0,38 (\pm 0,09) a
AGF+	2,08 (\pm 1,78) c	123,3 (\pm 40) b	1,18 (\pm 0,67) c	7,97 (\pm 10,32) c	24,09 (\pm 5,34) c	1,54 (\pm 0,81) c	0,23 (\pm 0,09) b

3.2 Plant morphology

Growth habit and covering rate. Growth habit, observed at the end of tillering period (BBCH=30) and at the beginning of booting (BBCH=41), is an indicator of the ability of the plant to cover the soil and eventually compete against weeds and avoid naked soil. We used a visual scale ranging from 1=erect habit to 9= prostrate, to characterize each variety in each treatment. Our question was: does the competition for water, space, light and nutrients that durum wheat varieties experienced in Agroforestry treatment influenced their plant habit? Table 9 shows that no significant difference was found between C and AGF treatment. But plant leaves were significantly more erected in AGF+ treatment than in C, with a maximal of 3. Between the two dates, the wheat has recovered in C and AGF treatments.

Table 9 Mean value of growth habit and covering rate noted of the 25 varieties in the three treatment in two crop growing moments: tillering (27/03/2017) and the beginning of booting (07/04/2017). Means with different letters are significantly different according to Tukey's HSD

	Growth habit mean noted 27/03/2017	Growth habit mean noted 07/04/2017	Covering rate mean noted 27/03/2017	Covering rate mean noted 07/04/2017
C	3,1 a	2,5 a [1-5]	3,4 b [2-4]	2,8 c [2-4]
AGF	3,3 a	2,6 a [1-5]	2,9 c [2-4]	3,1 b [2-5]
AGF+	1,4 b	1,7 b [1-3]	4,8 a [3-5]	4,5 a [3-5]

Among the genotypes, differences were noted. Some of them (cv. 45, 100, 101 and 534) were very early erected: they got a value of 1 (erect) in the 3 treatments for the two dates. Some others (cv. 6, 14 and 22), on the contrary, kept a value of 5 (half erect between the two dates of notations).

The covering rate was determined by observing the 2 central wheat rows of each plot. The data, showed significant differences between systems and high relation to the growth habit. Indeed, the erected plants of AGF+ treatment cannot cover the soil, thus leading to a bad covering rate: around 65 % of the ground soil is visible between rows. C got the lowest value in the second date of notation, which correspond to only 25 % of ground visible soil between rows. In the same date, AGF showed intermediate results with an average CR=3.1 (35% of visible ground soil between rows).

Number of tillers. The number of tiller per plant (Table 10) was decreased by 15 % in AGF treatment compared to control and by 31% in AGF+ treatment. These values are highly related to the coverage rate described above. C is the treatment which had the highest number of tiller per plant (2.42) and the highest covering rate (only 20 % of visible ground soil). AGF treatment showed intermediate results for both parameters and AGF+ with the lowest mean number of tillers per plant left 65% of ground soil naked.

Plant height evolution. Plant height was measured from the end of tillering till the end of flowering (Figure 13). For the first part of growing season, till BBCH=39 (flag leaf fully unrolled), plants in AGF treatment were higher than in C (+ 14 % in date 10/03/2017 and + 3%

till 24/03/2017). From the beginning of booting stage and till maturity plants in control treatments were higher (+5%) than in AGF treatment. Among genotypes a variability was observed.

Genotypes 11, 12, 266, 534 were the highest in each treatment and each date of notation (>100 cm the last date of notation). Genotypes 43 and 101 were the smallest in each treatment (< 65 cm the last date of notations).

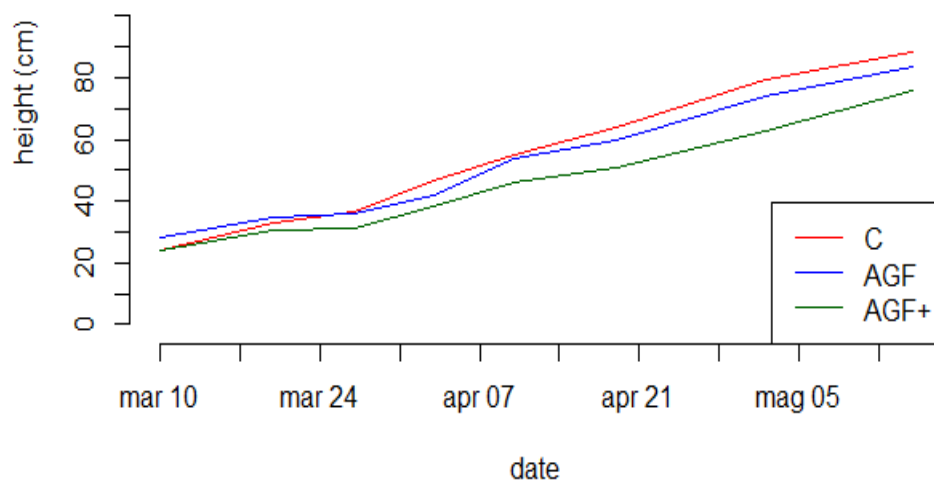


Figure 13 Dynamics of plant height from the end of tillering till the end of flowering. On the x axis, the date of plant height notation. On the y axis, the average height of all varieties plant for each treatment

Plant phenotyping at maturity. The control treatment showed the highest values compared to the two-agroforestry treatment considering plant height, spike length and spike and awn length (Table 10). Plant height was decreased by only 3 % in AGF treatment compared to C and did not show significant differences by HSD test. In AGF+ treatment plant height was significantly lower than in C (-20 %). The spike length and the spike + awn length were significantly different between treatments. Spike length was decreased by 23% in AGF treatment and by 41 % in AGF+; spike + awn length by 10% and 27 % respectively in the 2 agroforestry systems.

The distance between the flag leaf and the spike was the only morphological character for which AGF treatment reached a higher value compared to C (+3 %, even if not significantly different). AGF+ treatment, additionally, even if showing a distance decreased by 8 %, was not significantly different from the control.

Table 10 Morphology traits: *Tal_pl*= number of tillers per plant, plant height and spike characters in control, AGF and AGF+. Means (\pm S.E.) with different letters are significantly different according to Tukey's HSD

System	Tal_pl	Plant height (cm)	Spike length (cm)	Spike + awn length (cm)	Dist. from flag leaf to spike (cm)
C	2,42 (\pm 0,87) a	75,17 (\pm 15,76) a	6,02 (\pm 1,34) a	16,19 (\pm 1,86) a	18,17 (\pm 5,39) a
AGF	2,06 (\pm 0,47) b	72,84 (\pm 19,42) a	4,61 (\pm 1,00) b	14,55 (\pm 2,16) b	18,7 (\pm 4,69) a
AGF+	1,67 (\pm 0,72) c	60,28 (\pm 12,23) b	3,53 (\pm 0,75) c	11,85 (\pm 1,66) c	16,72 (\pm 4,35) a

2.3 Leaf Area Index

The average photosynthesis of crops grown under olive trees varied with light interception; data of the first chapter showed that PAR value was considerably reduced in agroforestry modalities (-33% for AGF, -55% for AGF+). Although LAI nominally means "leaf area index", we have to remember that the LAI-2200 we used is measuring all light-blocking objects, so "foliage area index" seems more appropriate. LAI is dimensionless, but it can be thought as m² one-sided foliage area/m² ground area.

Average LAI recorded during whole period were 1.67 LAI units for the control treatment, 1.51 for AGF and 0.76 for AGF+; significance was shown in the difference between systems by Tukey's HSD.

At BBCH=30, the first LAI recorded were 1,19 LAI units in full sun condition, 0.86 in AGF and 0.52 in AGF+. These values increased till reaching LAI max at the end of May; then it decreased because of leaf senescence. LAI max for AGF+ was recorded the 22/05/2017 with 1.19 units. Then this value started to decrease from the week after. LAI max in AGF and C was recorded one week after AGF+: in date 31/05/2017; LAI of AGF was 2.05 units and LAI of C treatment was 2.3 units (significantly different according to Tukey's HSD).

Foliage orientation. LAI-2200 is also giving the value of MTA (Mean Tip Angle) and DIFN (Diffuse non-interceptance). The value of MTA answers to “How is the foliage oriented?”. If all the foliage were horizontal, then the correct MTA would be 0°; if all were vertical, MTA would be 90°. Thus, we expect to see that its value is correlated with the data of growth habit, covering rate and number of tillers per plant presented above (Table 9 and 10).

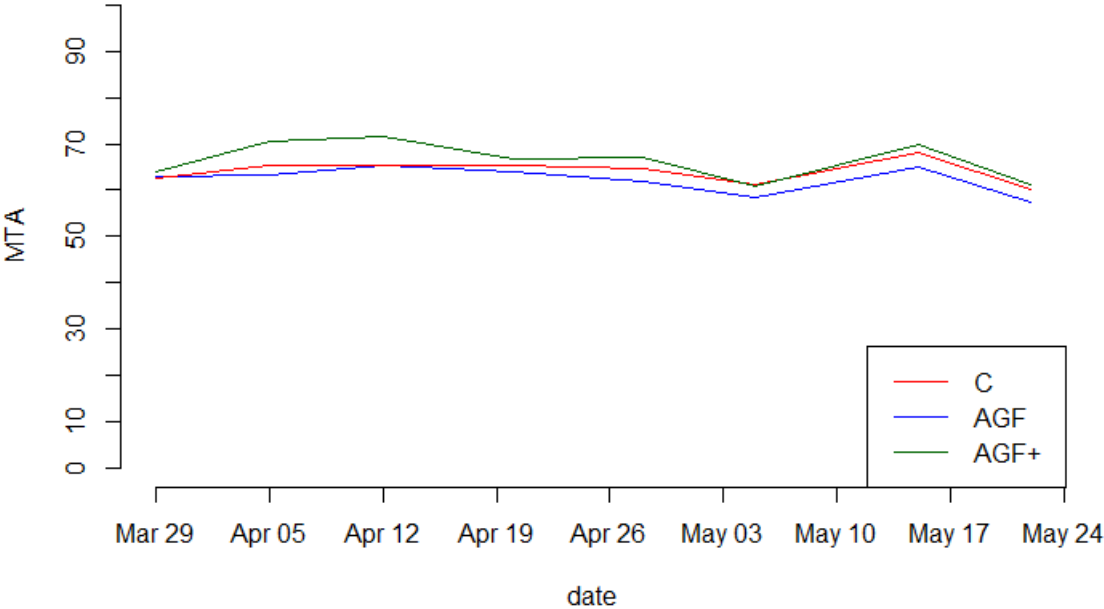


Figure 14 Mean MTA (Mean Tip Angle) value of the 25 genotypes in each treatment from the end of March till mid-May recorded weekly by means of LAI-2200.

Figure 14 shows the mean MTA value of 25 genotypes in each treatment from the end of March till mid-May. AGF+ treatment had the foliage with the more vertical orientation during all date of notations (except for the 05/05/2017). The control treatment showed an intermediate orientation of foliage. AGF showed the foliage more horizontally oriented for all dates of notation. If we focus on data recorded 05/04/2017, they are highly correlated with the growth habit noted two days after (Table 9). AGF+ treatment, with the most erect growth habit (1.7), had MTA=70.5 which means that his foliage created an angle of 70.5° with the soil. The control showed intermediate values. AGF treatment, which showed the more prostrate growth habit on 07/04/2017, had the lower MTA value with an angle of 62 ° between the foliage and the ground soil.

The value of DIFN (giving by LAI-2200) combines LAI and MTA into one number. It ranges from 0 (no sky visible to the sensor) to 1 (no foliage visible to the sensor) and is an indicator of “canopy light absorption”. DIFN recorded the first week of April are highly correlated with the

covering rate noted on 07/04/2017. DIFN values in this date for AGF+, AGF and C were 0.67, 0.39 and 0.37 respectively; the % of visible ground soil arise from covering rate notations are very similar to these values (64%, 35% and 25 % respectively).

Leaf area index is the most widely used parameter to investigate plant morphology within agroforestry literature. Previous studies mainly focused on testing how shade affects the spatial distribution of leaf area. Dufour et al. (2013) found that the reduction in crop LAI under low solar radiation conditions was partially compensated by increases in the fraction of the top and bottom leaf area to the total leaf area, which facilitated the interception of more solar radiation by the canopy. The same result was found by Mu et al. (2010): the fraction of leaf area of the top leaf layer (0.8–1.0 m above ground, mainly consisting of spikes and flag leaves) increased 0.8–2.1%, and the fraction of the bottom leaf layer (0–0.4 m) increased 0–6.1 %, while fraction of the mid leaf layer (0.4–0.8 m, mainly consisting of the top three leaves) reduced by 4.4–9.6 % under shading.

For the both papers this modified leaf are distribution under shade resulted in a more vertical distribution of LAI in wheat plants to better intercept light and compensate for low incident radiation. In our study, we observed that plants grown under the most shaded treatment (AGF+, -55% PAR) developed a canopy strongly oriented to the vertical. But the AGF treatment in our study, affected by an intermediate light reduction (-33%), reacted differently. Its growth habit, in fact, was not completely oriented to the vertical as AGF+: on the contrary, it was the most prostrate between treatment (growth habit=3.3 vs. AGF+=1.7 and C= 3.1). Moreover, it is the treatment which increased the most (+ 3% compared to control) the distance between the flag leaf and the spike. We can affirm, then, that it could distribute his biomass more strategically than AGF+: with a more prostrate orientation of foliage and a higher development of the main stem, plants were looking for light interception both covering the inter-row and growing higher.

As it was observed in previous studies (Mu et al. (2010), Artru et al. (2017), Li et al. (2010), despite canopy size decreased within agroforestry (LAI decreased by 10 % in AFGF compared to control), it is the distribution of leaf area in the different part of the plant which is strategical.

3.4 Leaf chlorophyll content

The amount of chlorophyll present in the plant leaves can be approached by the values measured thanks to the chlorophyll meter SPAD-502 (Hoel et al. 1998; Chang et al. 2003). SPAD readings are calculated based on two transmission values: the transmission of red light at 650 nm, which is absorbed by chlorophyll, and the transmission of infrared light at 940 nm, at which no chlorophyll absorption occurs.

From the beginning of recording, the last week of April, average SPAD values measured in the treatments were: 36.5 SPAD units in C, 31.4 in AGF and 29.9 in AGF+ (significantly different according to Tukey's HSD). These values were ranked in the same order till the end of April: BBCH=69 (Figure 15). Then, from the last days of April till the first week of May SPAD values decreased in all treatments. No significance was seen in the difference between the 2 agroforestry systems at this time, with chlorophyll content in C being significantly higher compared to both AGF systems. During this period, full flowering (BBCH=69) was reached within the 3 systems. These values recorded at the beginning of May remained constant for a variable time between systems till the beginning of leaf senescence as nutrients relocation to spikes.

The C treatment is the first where this decreasing was observed: average 10 days after the end of flowering, from mid-May, its value started a progressive decreasing. Agroforestry systems maintained the chlorophyll content observed at the beginning of May for a longer period: 18-20 days after the end of flowering AGF and AGF+ began to decrease progressively their chlorophyll content, AGF being faster than AGF+. The last recording date was the 31/05/2017 and it corresponded to grain filling-ripening phenological stage (BBCH 70-80). The C treatment showed the highest decrease at this date with 8.4 SPAD units. AGF chlorophyll content was still on average 13.02 and AGF+ showed the lowest decreased with average 25.3 SPAD units.

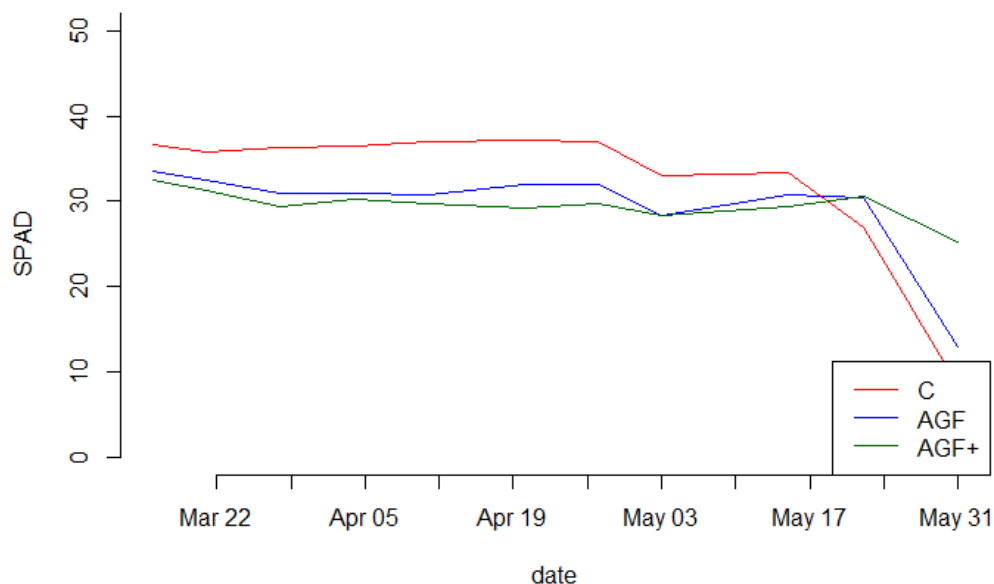


Figure 158 The average SPAD units for each treatment recorded weekly from 16/03/2017 till 31/05/2017

3.5 Quality parameters

The average percentage of grains with vitreous aspect was low (40%) compared to required value of 80%. The AGF+ showed significant higher % of grains with vitreous aspect compared to the AGF treatment and the control (+40 %).

The average protein content of control treatment was low (9.4%) compared to required values of 12-13%. The 2 agroforestry treatments had significantly higher % of proteins in the grains, +16% for AGF treatment and + 36 % for AGF+ (Table 11). The proteins composition was investigated more deeply thanks to SE-HPLC analyses. Protein rate was split into structural/metabolic (non-gluten) and storage proteins (gluten). Structural/metabolic proteins consisting of albumin, globulin and amphiphilic proteins (F5 fraction) were significantly more abundant in control treatment than in agroforestry system.

Wheat storage proteins, also called prolamins because of their high content of the amino acids proline and glutamine, is divided into three groups: sulphur-rich (F4), sulphur-poor (F2, F3) and high molecular weight glutenin subunits (F1). Gluten, furthermore, is divided in two forms of fraction: glutenins and gliadins. Glutenins are present in 3 mains categories: LMW-glutenins (F2), HMW-glutenins (F1) and the very HMW polymeric protein that only sonification can extract (Fi). Gliadins are classified in α , β , γ gliadins which are the Sulphur-poor fraction (F3) and ω gliadins which are the Sulphur-rich part (F4).

The ratio between structural and storage proteins in the 3 treatments, shown in Table 11, was on average 80% for storage proteins. AGF+ treatment had the higher values of storage proteins compared to full sun conditions. Within storage proteins, then, we observed that the abundance of glutenins and gliadins was different in treatments. Agroforestry treatments showed a significantly higher quantity of gliadins compared to control with +5% for AGF and +12% for AGF+. Consequently, looking at the gliadins/glutenins ratio within storage proteins, it was 1.31 in AGF+ treatment, 1.23 in AGF and 1.16 in C treatment.

Finally, the ratio of SDS-unextractable polymeric proteins (UPP) to total glutenin proteins was found significantly higher in C treatment compared to AGF treatment.

The higher protein content we found in agroforestry treatments agree with literature. Artru et al. (2017) and Dufour et al. (2013) observed an increase of protein content with increasing shade density (by up to 38% for artificial shade). According to them, the protein content of the grain resulted from the remobilization of N accumulated by the plant and is negatively correlated with final grain yield due to a dilution effect. Indeed, as they found, the increase in protein

concentration did not compensate the final yield decrease (47 % of yield decrease, 16% of protein content increase considering AGF). Observing each variety performances will let to better make evaluations.

The interest in investigating deeply the proteins composition is related to recent studies of gluten-related disorders and especially the Non Coeliac Gluten Sensitivity. Gluten, literally, is the major protein in the flour composition which acts for the dough strength (Susanna and Prabhasankar, 2013). There is still a lot to understand over which protein fraction is responsible for gluten sensitivity and there is the need to identify the main factors among varieties, cropping systems and process, that could have an influence on the amount of gluten and on gluten compositions.

Table 11 Quality parameters: the % of vitreous grains resulted, the protein content in the grains dry matter and then the protein composition: the proportion of structural and storage proteins within the whole protein content, the proportion of gliadin within storage proteins, and the UPP: the ratio of SDS-unextractable glutenin proteins

	vitreous grains %	Proteins % dry matter	structural proteines % /total proteines	storage proteins %/total proteins	gliadine %/total proteins	Fi
C	35,76 c	9,36 (±0,7)c	21,04 (±1,2) a	78,96 (±1,3) c	42,1 c	9,62 (±2,2) a
AGF	30 b	11,64 (±1,2) b	19,27 (±1,3) b	80,73 (±1,3) b	44,23 b	7,95 (±2,2) b
AGF+	55 a	14,58 (±1,9) a	14,44 (±2,1) c	81,56 (±2,1) a	46,09 a	8,55 (±2,4) ab

4. Conclusions

In this paper, we investigated the impact of Agroforestry on durum wheat yield and morphology by considering the 25 genotypes tested as random effect of the environment. The results presented, thus, are hiding the variability of durum wheat genetic profile tested: from pure lines modern varieties till ancient populations. By investigating deeply this genetic variability, we might be able to identify the morphological traits of varieties with better performances within agroforestry conditions. If the varieties tested will let this, we might be able to define the ideotype of durum wheat variety adapted to agroforestry.

Moreover, what we observed in our study is that in shade conditions, grains had a higher quantity of storage proteins (gluten), but a higher gliadins/glutenins ratio within gluten composition. It will be interesting to better understand the potential of agroforestry farming in modifying protein composition of the cereal understored.

III. BREEDING FOR AGROFORESTRY: LOOKING FOR AN APPROPRIATE TEST

1. Introduction

Several authors, assessing light competition in temperate agroforestry systems, concluded that the success of agroforestry depends on the selection of shade-tolerant species (Artru et al. 2017; Ehret et al., 2015; Friday et al., 2002; Barro et al., 2012). According to Athanasiou et al. (2010), when plants are grown under a particular set of conditions they adjust their photosynthetic capacity to match those conditions. As most of the crop species currently used were selected in full light conditions, crop breeding programs looking for photoacclimation traits are necessary to select shade tolerant cultivars, adapted to agroforestry (Retkute et al. 2015; Li et al. 2010).

As radiation is likely to be the principal limiting resource for understorey crops (Artru et al. 2017), previous studies tested the use of artificial shade system on crop growth and yield by means of different shading materials and for a variable period (periodic vs. continuous). These test design aimed to differentiate the effect of light from other belowground interactions (Friday et al. 2015). Artru et al. (2017) monitored winter wheat growth and productivity under artificial shade provided by camouflage shade-netting, in the aim to reproduce a rapidly fluctuating sun/shade pattern. Varella et al. (2011) investigated if wooden slatted structures reproduced well the daily periodic light fluctuation and the spectral composition observed under trees in comparison with conventional plastic shade-cloth. In Dufour et al. (2013) experiment, in order to mimic the increasing leaf area of walnut trees, they add overlapping shade cloth during durum wheat growing season.

The success of the selection process, according to Varella et al. (2011), is therefore dependent on the accuracy with which the artificial shade mimics the light environment and the plant responses to them. The aim of the present work was to assess the appropriateness of a permanent shading cloth over durum wheat plants grown in pots to be used as pre-breeding test for selecting shade-tolerant genotypes. Does it mimic the same shade effects determined by olive trees rows in an agroforestry system?

Moreover, the examples in literature are breeding test realized in the field thus needing a great surface and limiting the number of varieties that can be tested in the same time. The main idea

in this study was to evaluate a breeding test which allow to screen a great number of varieties for their adaptation to shade in a limited surface area.

2. Materials and methods

The experiments were conducted at INRA station DIASCOPE in Manguio (43° 35' N, 3° 45' E) in 2017. 25 genotypes of durum wheat were tested in 2 experimental trials:

- In the field, where natural shade was provided by the olive trees in the alley cropping design
- In a greenhouse, where two modalities were implemented: “full-sun” and artificial shade

2.1 Experimental design

Field trial was designed as described in (paragraph I.2.2) The “pre – breeding test” was implemented inside a greenhouse. The greenhouse was of multi-chapel glass type (OPTILUX 9.60 m) with rigid PVC walls (ONDEX Bio 2 Cristal) and a ground surface of 83 m². Inside the greenhouse, 25 durum wheat varieties were cultivated in pots (one plant/pot) and subjected to 2 treatments: Control (C) and Shade (S). Shade effect was created by putting pots in a cover shelter from sowing to harvesting (Figure 16). The design consisted in 3 repetitions (pots) per variety sown in each treatment thus obtaining 75 pots in the Control treatment and 75 pots in the Shade treatment.

Each pot was filled with 5 L of soil and 3 seeds were sown per pot at 25/02/2017. Before tillering (BBCH=20) only one plant per pot was kept and the other 2 were manually removed. An irrigation system (capacity of 2 liters/hour) run 10 minutes per 2 days/week from sowing to harvesting. Neither fertilizer neither chemicals were used, as in the field trial.

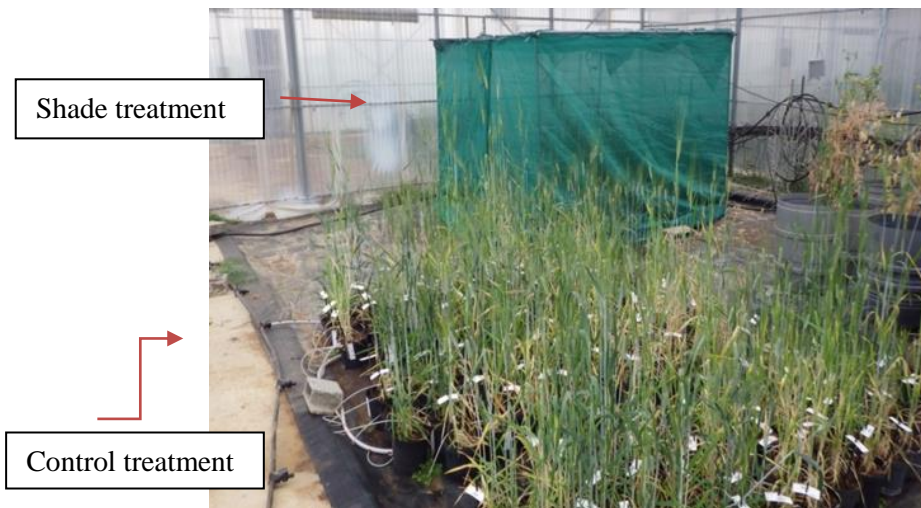
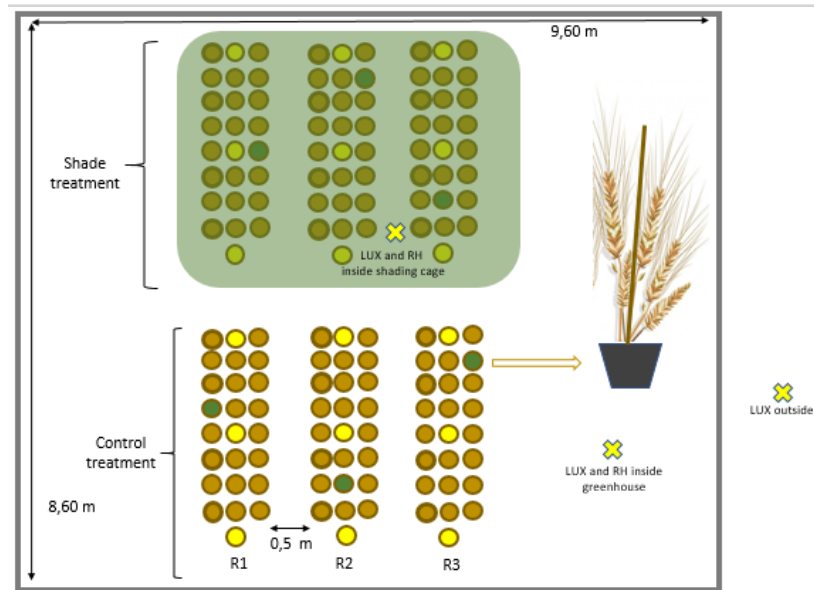


Figure 16. Design of the greenhouse treatment (on the top), and a photo of the control treatment and the shade treatment in the greenhouse (on the bottom).

2.2 Environmental parameters

Light and air relative humidity were recorded by means of portable Luxmeter (Voltcraft – DT 8820, working range 0-2000 lux, 25-95% HR ($\pm 5\%$)) twice a week from tillering (BBCH=20) till the end of anthesis. The LUX and RH recordings was done 3 times per day: in the morning (8:00-9:00 am) at midday (12:00-1:00 pm) and in the evening (4:00-5:00 pm), in the greenhouse at defined geographical position (Figure 14) and outside. The differences between LUX and RH data were averaged per day.

The maximal temperature threshold of the greenhouse was set at 25 °C during day hours and at 22 °C during night hours: overpassed these thresholds a cooling system began to run.

2.3 Plant phenotyping during the growing season

Wheat phenology was recorded weekly in the same dates and with the same materials and methods than the field trial as described in chapter II - paragraph 2.1.

2.4 Plant phenotyping at maturity

One week before field harvesting, each durum wheat plant in the greenhouse was cut at stem collet level and then submitted to several measures, as described for the field trial.

Among them: Morphology traits (height of the plant, spike length, awn length, distance between flag leaf and base of the spike), and reproductive traits (number of spikes, weight of 1000 grains (TGW), number of seeds/spike, biomass and harvest index) were determined as in chapter II - paragraph 2.5.

3. Results and discussions

3.1 Environmental conditions: comparison between greenhouse and fields

The difference between LUX data recorded outside and those recorded inside greenhouse allows to quantify the effect of greenhouse walls compared to full sun conditions. This difference is about 6% (1542 LUX is the daily average recorded outside and 1446 LUX is the one inside) with a maximum of -8% at midday.

Inside the greenhouse, the difference between control and shade treatments was about 57 %. This difference varies during the day according to the hours (Figure 17). The main difference was noticed at 8-9 am with 68 % of reduction under the cover shelter compared to sunny greenhouse conditions.

In the field trial, the reduction of PAR was 33 % in AGF and 55% in AGF+ compared to the full sun control. This value is closed to the level of reduction between “control” treatment and “shade” treatment in the greenhouse.

HR was not significantly different between the 2 treatments in the greenhouse: at 8-9 am, the average HR was 66 % for control conditions and 67% inside the cover-shelter; at midday, it was average 46 % for the both and at 4-5 pm it was average 48 % for the both.

In the field trial, there was a higher difference between control and agroforestry treatments: at 8-9 am in AGF HR was 7% higher than in the control, at midday HR was the same in the two treatment and at 4-5 pm HR was higher in the control of about 5 %.

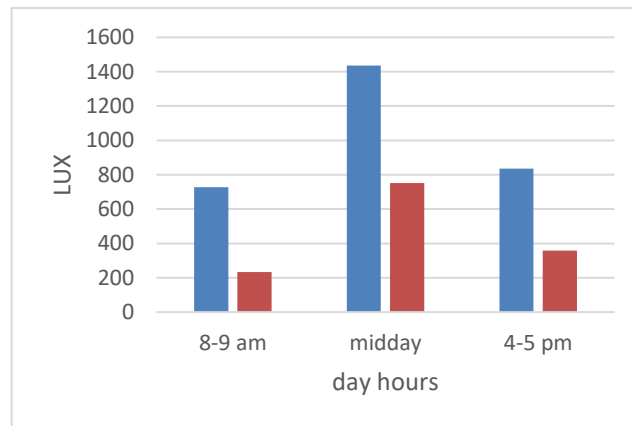


Figure 17 Average LUX recorded in the greenhouse for the 3 day hours at which recording were computed (blue: full sun; red: shaded).

3.2 Plant phenotyping during the growing season

The plants subjected to the 2 Control treatment in field and in greenhouse did not achieved phenological stages at the same time (Figure 18). For instance, BBCH=39 (flag leaf fully unrolled) was reached in greenhouse Control 10 days before the field Control, BBCH=50 (beginning of heading) 7 days before, and BBCH=69 (end of anthesis) 10 days before. It seems that the difference of temperature (maintained in the greenhouse around 25°C) may explain the differences in growing speed.

The difference between the control and the shade treatments in terms of phenology is greater than in the field. For instance, the stage BBCH=39 was reached, in the greenhouse shade conditions, 14 days after control treatment. In the field, during the period between BBCH=39 and maturity, phenology of plants was only delayed by 2-4 days as maximum in AGF treatment and by 5-7 days in AGF+ compared to Control. Even if the level of light intensity is comparable between greenhouse-shade-treatment and field-AGF+ treatment, the plants reached the different stages and maturity earlier in the field. But it is difficult to compare between greenhouse and fields as the date of sowing were not the same (2 months of gap) and therefore photoperiod was also different.

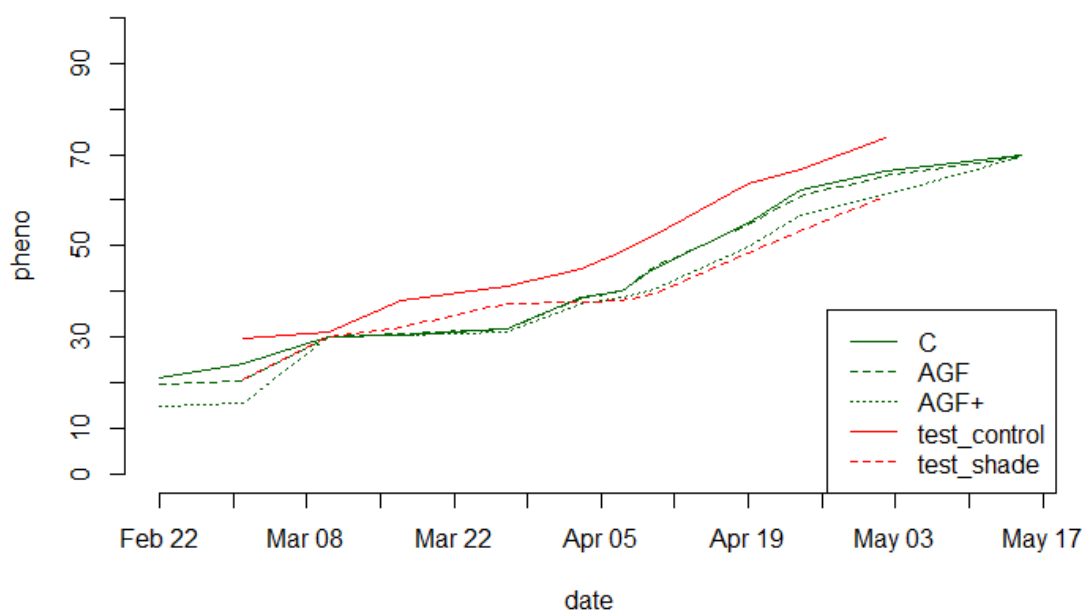


Figure 18 The phenological development of durum wheat in the treatments: the three treatments in the field: the control, AGF and AGF+ and the two treatments in the greenhouse: the control and the shade treatment. Date of phenology notations on x axis, and BBCH stage noted in y axis.

3.3 Yield components

Greenhouse treatments. Yield components obtained in the greenhouse-shade treatment were significantly lower than those obtained in the control treatment (Table 12). Among them, the number of spikes per plant (one plant means one seed sown) and the biomass showed the highest percentages of reduction (-45% and -56 % respectively). Harvest index was 0.42 in both treatments.

Table 12 Yield components (means of 25 varieties \pm SD) obtained in the two greenhouse treatments. Spike_pl=number of spikes per plant (one plant means one seed sown), gr_spike=number of grains per spike, TGW=Thousand grains weight, biomass and HI=harvest index. Means with different letters are significantly different according to Tukey's HSD.

		spike_pl	biomasse	gr_spike	PMG	HI
Greenhouse	control	10,08 (7,1) a	49,94 (31) a	41,37 (15,4) a	52,92 (9,5) a	0,42 (0,01) a
	shade	5,98 (6,1) b	20,93 (12,4) b	38,39 (12,9) b	45,72 (7,4) b	0,42 (0,1) a
% variation	Shade-Control	-45%	-56%	-6%	-14%	-0,08%

Comparison with field results. The level of yield components in the greenhouse was higher than those measured in the field (Table 13). This evidence seems due to the differences in the growing conditions: in the greenhouse, each durum wheat plant was grown in a single pot, separated from the others, while in the field the crop was sown in the same medium.

Consequently, avoiding competition with other plants allows the 2 treatments in the greenhouse achieving higher yield components). As water was not found as limiting factor in agroforestry conditions we assume that irrigation system in the greenhouse was not a factor increasing productivity compared to field conditions.

Table 13. Percentage of yield component variation between the greenhouse-shade treatment and the greenhouse-control treatment and between the two field-shade and the field-control treatments. Means with different letters are significantly different according to Tukey's HSD.

		spike_pl % variation	biomass % variation	gr_spike % variation	TGW % variation	HI % variation
Greenhouse	Shade - Control	-45% (±22) a	-56% (±2) b	-6% (±28) b	-14% (±13) b	-0,08% (±1) b
	AGF+ - C	-49% (±21) a	-79% (±1) a	-56% (±47) a	-54% (±9) a	-40% (±2) a
Field	AGF - C	-26% (±26) b	-49% (±24) b	-28% (±28) b	-4% (±4) c	-3% (±1) b

The % of reduction of plant biomass and HI in the greenhouse between shade and control treatment were not significantly different from those of AGF treatment compared to C treatment in the field trial. For the number of spike per plant, the reduction found in the greenhouse is close to that observed for AGF+ in the field. Moreover, in greenhouse TGW was affected in higher proportion compared to AGF treatment in the field (-14% vs. -4%).

3.4 Plant phenotyping at maturity

Greenhouse treatments. Plant height measured for the greenhouse-shade treatment showed a tendency to increase (+3%) compared to the one measured in the control treatment (even if not significance was seen between the treatments by Tukey's test (Table 14). The distance between the flag leaf to the spike was significantly higher in plants submitted to the shade treatment with an increase of 24%. Then, spike length and spike and awn length were decreased by shade treatment (significantly and not significantly, respectively).

Table 14. Morphological traits of plants in the two greenhouse treatments. Means (\pm S.E.) with different letters are significantly different according to Tukey's HSD test.

		Plant height (cm)	Spike length (cm)	Spike and awn length (cm)	Flag leaf to spike distance (cm)
Greenhouse	Control	91,9 (\pm 31) a	8,72 (\pm 2,1) a	20,29 (\pm 3,5) a	17,62 (\pm 7,9) b
	Shade	95,6 (\pm 32) a	7,6 (\pm 1,4) b	19,28 (\pm 2,8) a	20,91 (\pm 8,2) a
% variation	Shade-Control	+ 5%	-11%	-4%	+ 24%

Comparison with field results. Plant height, spike length and spike and awn length in the field-control treatment (Table 15) were decreased by average 23% compared to greenhouse-control treatment. Among them, the highest decreased was seen in the spike length with a 31% of reduction from the greenhouse to the field control treatment. On the contrary, the distance from flag leaf to spike was higher in the field-control (+3%) compared to the greenhouse-control. Then, comparing the morphological traits measured in the greenhouse-shade treatment and in the field AGF-shade treatment an average decrease of 25% was seen for the AGF-shade treatment. As for the controls treatments, the highest reduction was for the spike length, decreased by 39 % in AGF-shade treatment compared to the greenhouse-shade treatment.

The % of reduction between the control treatments and the shade treatments in the greenhouse and in the field, considering plant height and spike traits, were higher in the field compared to the greenhouse (Table 15). The impact of shade treatment on the plant height was an increase (+5%) in the greenhouse-shade treatment and a decrease in the two field- shade treatment (very low in AGF treatment with -3 % and higher in AGF+ with -19 %), compared to the greenhouse- and the field-control respectively. Then, considering the distance between the flag leaf and the spike, both green-house-shade treatment and AGF-shade treatment show an increase compared to the respective control treatments. This increase was higher in the greenhouse (+24%) than in the field (AGF + 5% compared to C treatment). The % of reduction of spike length in the shade treatments compared to controls was significantly different in the greenhouse and in the field (% reduction of greenhouse shade closer to the one in AGF than to the one in AGF+ treatment).

Table 15. Percentage variation of morphological traits between the greenhouse-shade treatment and the greenhouse-control treatment and between the two field-shade treatment and the field-control treatment. Means with different letters are significantly different according to Tukey's HSD.

		Plant height (cm) % variation	Spike length (cm) % variation	Spike and awn length (cm) % variation	Dist from flag leaf to spike (cm) % variation
Greenhouse	Shade - Control	+ 5% (±0,1) c	-11% (±0,1) c	-4% (±0,06) b	+ 24% (±0,38) b
	AGF+ - C	-19% (±0,1) a	-40% (±0,1) a	-26% (±0,1) a	-6% (±0,1) a
Field	AGF - C	-3% (±0,1) b	-22% (±0,1) b	-9% (±0,1) b	+ 5% (±0,1) a

3.5 Pre-test for shade-adapted varieties

The grain dry matter produced per plant was used to evaluate the variability among genotypes. In the greenhouse-shade treatment, the 25 genotypes averagely produced 8.4 g of grains (dry matter) per plant (plant means one seed: in every pot there was one seed) (Table 16). This value was 60% lower compared to 21.9 g achieved in the greenhouse-control treatment. In the field the grains produced per plant (grams of dry matter) were 1.4 g in the AGF-shade treatment and 2.7 g in the Control. Considering all genotypes the % of reduction of this yield component was higher between greenhouse-shade and greenhouse-control treatment than between field-AGF and field-control treatment.

Table 16. Grain dry matter produced (in grams) per plant as average of all genotypes tested in the greenhouse and the field. Pl=plant (one plant=one seed in each pot).

	Greenhouse			Field		
	Shade grains/plant grams	Control grains/plant grams	% variation	AGF - Shade grains/plant grams	Control grains/plant grams	% variation
mean of all genotypes	8,4	21,9	-60%	1,4	2,7	-44%

According to the grams of grains produced per plant in the greenhouse-shade treatment we define then two classes of genotypes. The genotypes which produced more than 10 grams of grains per plant and the genotypes which produced less than 8 grams of grains per plant. The first class, then would contain the genotypes which might be more tolerant to shade conditions and adapted to agroforestry. The second class would include the genotypes which yield was highly reduced by shade and might be not adapted to agroforestry. Then, we investigated the performances showed by these genotypes in the field trial.

Considering the first class of genotypes (Table 17), var. 6, 57, 59 and 56 showed an average 36% of reduction in the greenhouse-shade treatment compared to the greenhouse-control treatment. Among them, the var. 56 reached in the greenhouse-control a lower yield compared to shade conditions and reached in the greenhouse-shade treatment the highest yield. The same 4 genotypes in the field showed an average % of decrease in the AGF-shade treatment of 46% compared to the Control. Among them, var 56 still obtained the highest yield in the field shade and showed a % of decrease under shade below the average for this class.

Table 17 First class of genotypes: the threshold was a grains dry matter per plant > 10 grams (one plant means one seed; one seed in each pot) produced in the greenhouse shade treatment. These 4 genotypes are then described for the grain dry matter reached in the greenhouse-control treatment and in the three field treatments.

Genotypes	Greenhouse			Field		
	Shade dm/pl	Control dm/pl	% variation	AGF- Shade dm/pl	Control dm/pl	% variation
56	13,9	12,4	+12%	2,56	4,16	-38%
6	11,6	21	-45%	0,93	2,59	-64%
57	11,6	25,3	-54%	1,99	2,69	-26%
59	10,3	23,6	-57%	1,04	2,41	-57%
mean	11,8	20,56	-36%	1,63	2,96	-46%

Then, the class of genotypes reaching lower than 8 grams of grains dry matter per plant contained 4 genotypes which produced in the greenhouse-shade treatment on average 6.35 g (Table 18). The % of decrease from the yield reached in the greenhouse-control treatment was of 79%. These same varieties showed in the field a reduction percentage of 63% in the AGF-shade treatment compared to the field-control.

Table 18 Second class of genotypes: the threshold was a grain dry matter per plant < 8 grams (one plant means one seed; one seed in each pot) produced in the greenhouse shade treatment. These 4 genotypes are then described for the grains dry matter reached in the greenhouse-control treatment and in the three field treatments.

Genotypes	Greenhouse			Field		
	Shade dm/pl	Control dm/pl	% variation	AGF- Shade dm/pl	Control dm/pl	% variation
101	6,56	23,48	72	1,28	3,08	59
9	8,02	33,98	76	0,81	3,47	77
55	6,24	30,87	80	0,69	1,55	56
12	4,58	32,79	86	1,59	3,97	60
mean	6,35	30,28	79	1,09	3,02	63

4. Discussion

Decreased crop yield is a constant result in agroforestry farming. As farmer's income depends mainly on yield (and quality) performances, the spreading of agroforestry farming relies on the provision of cultivars maintaining sustainable yield performances. Researchers' concern, thus, is increasing over the necessity to develop appropriate breeding programs looking for agroforestry suitable varieties. As light reduction is widely considered the main factor decreasing yield in this context, previous research was addressed in designing tests where crops were grown with only light as limiting factor (Varella et al. 2011; Dufour et al. 2013; Li et al. 2008). Therefore, they evaluated the accuracy of different artificial shade material, techniques and periods to simulate trees shade effect.

In our study, we were looking for an appropriate test design that simulated the shade effect of olive tree rows and that could allow to potentially test the performances of a wide number of cultivars. Therefore, the choice of a cover shelter which permanently shade durum wheat was in the willing to simulate the effect of a tree species with evergreen leaves as the olive tree. Additionally, the use of pots allowed us to place the test in reduced space compared to open fields withplots. Particularly, the choice of growing each plant in a single pot allowed not only to avoid the belowground interaction with olive tree, but also to avoid any effect due to competition with neighbor durum wheat plant. In this way, the factor "light" was isolated from all possible belowground interaction.

The permanent cover shelter in our greenhouse determined a light reduction on average of 57% compared to control, which was similar to the reduction we found in AGF+ treatment in the field agroforestry. Even if not the same tool was used to assess light parameter (a pyranometer recording photon quantity in PAR units in the field and a LUX meter measuring LUX units in greenhouse), the % of reduction between the control treatment and the shade treatment in the test were similar to the ones in the field.

The presence of the greenhouse walls determined a reduction of 6 % in LUX units compared to outside radiation. Further studies aiming to assess the effect on quality of light reaching plants will allow to better understand the greenhouse effect. Li et al. (2008) pointed out the importance of considering the effect of agroforestry not only in reducing radiation but also in altering the spectral quality. According to these authors, with increasing intensity of shading, the fraction of blue light increases while the one of red light decrease which might affect physiological (carbon use efficiency) and morphological crop characters. Consequently, to investigate the effect of artificial shade materials over radiation quality is essential in determining its accuracy in simulating the tree effects.

As stated by Varella et al. (2011), the accuracy of a pre-test for varieties adapted to agroforestry relies on simulating tree characteristics in terms of canopy size and phenology and mimicking the crop responses to them. In our experiment, we observed at which percentages yield components and morphology traits were reduced by artificial shade in the greenhouse and by natural olive tree shade in the field. Considering biomass, HI and yield components, similar ($P>0.05$) % of reductions were observed in greenhouse and in AGF treatment (33% PAR reduction). According to Li et al (2008), this is due to the potential of shade to improve the redistribution of storage dry matter. The number of spike/plant was the most affected (reduced) yield component in the greenhouse – shade treatment. A similar percentage of reduction was measured in AGF+-shade treatment (the field treatment with the highest light reduction: -55% PAR).

Considering morphology, durum wheat showed a higher tendency to increase its height and extend the distance between flag leaf and spike under shade in greenhouse compared to the field. According to Valladares et al. (2002) and Li et al (2008), higher tolerance to low light conditions can be achieved by enhanced plasticity of “light-capturing” plant growing. In our experiment olive tree shade increased the distance between the flag leaf and the spike by 5% and the artificial shade in the greenhouse increased it by 24%. Plant height, additionally, was decreased by 3% by AGF shade treatment in the field, but was slightly (+3%) increased by artificial shade in the greenhouse. We could deduce that it is not only light reduction to

determine the magnitude of the effect on morphology. In the field, we did not observe this “elongation” behavior at the same extent as in the greenhouse, probably because plant growth could have been limited by other factors, like competition with other durum wheat plants or competition with olive trees could have reduced this common tendency of plants in shaded environment.

Dufour et al. (2013), while testing an artificial shading structure simulating the effect of walnut trees over a durum wheat crop understored, found that the reduction of final yield was higher (-20%) under the real agroforestry treatment than under the artificial shade (-16%).

V. IMPACT OF ASSOCIATED FIELD CROPS ON ORGANIC OLIVE ORCHARD PRODUCTION

1. Introduction

Olives and olive oil are the key basis in the healthy Mediterranean diet and there is an increase demand for such products coming from sustainable and organic farming (Afidol, 2015). Most often organic orchards are zero input ancient orchards located in extensive hilly and mountainous areas susceptible to soil erosion (Taguas et al., 2010). These low-density orchards present a low productivity and therefore are progressively abandoned (EU Olivero project, 2007). High-density olive orchards have been spreading over flat Mediterranean regions in order to get advantages from fertile lands and better condition for agricultural practices (Pastor et al. 2007).

However, these new orchards commonly need of chemical treatments and are therefore not really compatible with the organic regulation. Moreover, despite the increasing production, this system does not always ensure better farm profitability because of the increasing volatility of olive oil market prices and because of the fruit-bearing alternance.

Traditional or high-yielding olive orchards present most often large space between tree rows (5 m to 9 m). To face the above issues and also the increase need for (i) arable land use optimization, (ii) sun radiation use maximisation and (iii) erosion limitation, sowing an associated crop could be a relevant solution.

As organic durum wheat and chickpea are also typical Mediterranean crops, cultivated over the same environmental conditions of olive trees, they represent interesting candidates to be associated crops. But this Agroforestry system raises some questions such as:

- Is it possible to grow field crops in an abandoned olive orchard without ploughing and usual soil preparation to avoid olive root damages?
- What is the impact on olive production?
- Does this agroforestry system may produce additional income for the farmers?

The aim of this paper is to answer these questions and to analyse the sustainability of such agroforestry design.

2. Material and methods

2.1 Experimental design

The olive orchard object of this study is the AGF intercropping treatment (paragraph I.2.2).

160 olive trees (9 clones deriving from the cultivar Picholine) were planted in 2002 in a 6 x 6 m design and have never been pruned neither treated during the research project that ended in 2007. After that, the orchard has been abandoned until 2012 when it has been officially converted into organic and trees were intensively pruned for the first time in order to reconstruct the structure of plant canopy. From 2014 to 2017, trees have been yearly pruned during the spring period and olives have been hand-harvested at the beginning of November each year.

2.2 Organic farming

Orchard protection and grass strips management have been ensured by several agricultural practices respecting organic farming regulations. Against *Bactrocera Oleae*, the most relevant pest damaging olives, specific attractive traps have been located out of the orchard from the early season in order to limit the first-generation of the fly. Then, all along the season, traps captures were used to monitor the development of the pest population. Additionally, capture data were weekly sent to the Technical Center AFIDOL which elaborated them to suggest preventive practices within monitored territories (<http://afidol.org/oleiculteur/carte-des-piegeages/>). Once or maximum twice a year, a Kaolin-based particle film was spread to protect fruits from *B. oleae* fly. Furthermore 15 plants of *Inula viscosa* have been planted. This specie is known for being good host for beneficial insects which control the olive fruit-fly (Parolin et al., 2014).

2.3 Crop association management

25 durum wheat varieties in an annual rotation with legumes (chickpea, fababean, forage mix) (paragraph I.2.1) have been sown between olive trees rows at the beginning of November each year. In the respect of organic regulation, no treatment has been done to wheat for the all period, neither protection neither fertilization products. We assume, therefore, that nitrogen supply of the soil is driven by legumes action in the crop association.

Legumes crops used in the rotation were chickpea the first year, chickpea-faba beans the second and oat-vetch-clover mix the third. They have been sown at the end of winter, ground at the end of September and incorporated into the soil through harrowing.

Figure 19 shows the trial design in the orchard. We focused on the northern part (in blue), containing 9 clones of Picholine olive trees; crops were sown in the yellow part of the orchard and, in the green part, soil has never been ploughed neither harrowed and was always covered by natural grass. This green area represents the “Forest” control.



Figure 19 Olive orchard trial (in blue) corresponding to the AGF treatment: 8 rows with 12 plants/row. Yellow lines define areas where durum wheat plots in rotation with legume crops have been placed from 2014 to 2017. Green colour shows the control part of the experiment: olive trees stayed with grass covered ground soil from planting till present moment. (Source : Google earth Pro photo 2012)

2.4 Monitoring activities on olive trees

In the frame of a previous research project carried on from 2002 to 2007 (ONIOL, 2007), several data concerning vegetative development of each clone of Picholine have been collected: tree diameter, height, thickness in the row and between rows to assess vigor and volume, timing of production beginning, flowering rate, productivity (yield and size of olives) and degree of alternation.

Then, in the frame of the present work, olive trees have been monitored from 2014 to 2017. The BBCH (BASF, Bayer, Ciba-Geigy and Hoechst) scale has been used to describe the growth stage of olive tree (Lancashire et al., 1991; Zadoks et al., 1974). Flowering time was recorded

for each tree. Flower quantity was assessed through visual estimation in a 0-5 scale. Each year, the total amount of olive was weighed for each tree, and samples of 100 counted olives were weighed. The number of olives produced per tree was estimated from these data. The number of olives fallen on the soil surface was estimated visually and the number of olives with damage (holes) of *B. oleae* was counted.

2.5 Soil samples analyses

The materials and the methods are described in paragraph I.2.5.

2.6 Economic impact of association

Profitability of introducing durum wheat crop cultivation into an organic olive orchard has been evaluated. The economic analysis has been carried on for the two subparts of the system: additional income given by durum wheat selling and olive trees productivity.

The gross income of durum wheat production was calculated by multiplying the average yield of the 25 genotypes with the average quotation (2014-2017 period) of organic durum wheat in the South of France. Profit has been calculated by subtracting the cultivation costs from the gross income, and it was compared with the average yield found in the open-field control.

Concerning the olive production, the average yield registered in some zones of the yellow part (Figure 18) was compared with those registered in some zones showing similar fertility level in the green part (control). Evolution of the data over the 3 years of experiment has been considered. Level of prices used in the analysis arises from South of France organic olive orchard market.

System profitability has been calculated by adding additional incomes arising from selling of durum wheat grains produced and from the increase in olive tree productivity.

3. Results and discussion

3.1 Impact on olive production

The olive orchard showed a heterogeneous production according to space and time (Figure 20). In 2014, while intercropping was not yet implemented, olive production was highly variable between rows (from 219,8 g/tree for row A to 1416 g for row H) and between the Picholine clones (from 25.3 g for P30 to 247.8 g for P18). Zones of similar productivity (yield of olives produced per tree) in 2014 were determined and two zones were defined: the zone where trees produced averagely 400 g of olives per tree in 2014 and the zone where trees produced averagely 700 g of olives per tree in 2014.

A	B	C	D	E	F	G	H
P71	P30	P08	P35	P35	P21	P28	P28
P71	P71	P18	P28	P21	P49	P18	P21
P71	P30	P28	P35	P49	P30	P30	P18
P08	P18	P08	P35	P21	P28	P21	P35
P18	P21	P18	P28	P49	P08	P30	P08
P08	P71	P28	P71	P71	X	P66	P35
P18	P28	P30	P66	P30	P08	P18	P66
P71	P49	P21	P71	P21	P66	P30	P49
P49	P35	P71	P66	P49	P30	P49	P35
P49	X	X	P35	P30	P66	P18	P35
P08	P21	P18	X	P28	P08		

Legend

initial average productivity

400 g

700 g

Inter-row management

crop

grass

Figura 20 Picholine olive orchard. A to H columns correspond to the olive trees rows. Px defines the number of the Picholine clone as detailed by individual tree, and X refers to the absence of trees. Colours explained in the legend show tree rows with initial comparable olive productivity in 2014 and type of management in the ground soil at both sides of tree rows.

3.2 Variability between rows

2 zones having similar initial (2014) olive productivity were compared: 400 g/tree (grey color in Figure 18) and 700 g/tree (orange color in Figure 18) Figure 18 shows also the zones of Agroforestry (yellow) and those of forest control (Green).

The agroforestry zones starting with approximately 400 g/tree in 2014 (Table 19), reached an average of 2673 g/trees in 2015 and 3114 g/tree in 2016. Forest zone (zone 4) reached 1585 g/tree in 2015 and 1770 g/tree in 2016. Considering the whole period 2014-2016, then, the agroforestry zones increased their initial productivity (average 400 g/tree) by 7 times and the forest zone (control) by 3.2 times.

Zones showing a productivity comprised between 650 and 700 g/tree in 2014 (Table 20) resulted with an average of 1987 g/tree in 2015 and an average of 3278 g/tree in 2016. Forest zone (zone 7) reached 1692 g/tree in 2015 and 2645 g/tree in 2016. The increase of productivity over the 2014-2016 period was of 3.8 times the initial value for agroforestry zones (zones 5 and 6) and of 2.8 times the initial value in the control zone (zone 7).

The weight of 100 olives was greater in 2015 (average +10%) than in 2016, except for zone 7 (-3%). This result is due the lower yield in 2015.

Table 19 Productivity of trees with comparable average 400 g/tree yield in 2014. The 4 zones correspond to areas shown in grey in figure 2. Yield is defined by 2 yield components: olive Y/tree (in 2014, 2015, 2016), and weight of 100 olive (in 2015 and 2016). Data presented for each zone result from the average of the different olive tree situated in the same zone.

initial productivity 400 g/tree	row	inter-row	average yield / tree			average weight (fresh) of 100 olives	
			2014	2015	2016	2015	2016
zone 1	C	crop	426,3	2872,3	2027,6	429,1	403,7
zone 2	D	crop	387,3	1723	2850	531,9	496,6
zone 3	F	crop	340,9	3425,3	4465,7	482,7	454,1
zone 4	B	grass	425	1585	1770	494,3	424,8

Table 20 Productivity of trees with comparable average 700 g/tree yield in 2014. The 3 zones correspond to areas shown in orange in figure 2. Yield is defined by 2 of its components: olives Y/tree (in 2014, 2015, 2016); weight of 100 olive (in 2015 and 2016). Data presented for each zone result from the average of the different olive tree situated in the same zone.

initial productivity 700 g/tree	row	inter-row	average yield / tree			average weight (fresh) of 100 olives	
			2014	2015	2016	2015	2016
zone 5	A	crop	653,1	2162,5	2610,0	487,9	436,1
zone 6	D	crop	693,6	1813,1	3947,1	474,5	389,8
zone 7	G	grass	690,5	1692,1	2645,6	419,9	431,5

3.3 Variability between clones

All clones produced less than 250 g/tree in 2004 (Figure 21). In 2005 all clone reached more than 2000 g/tree, excepted for P21 and P30 which produced on average 1700 g/tree. Higher yield in 2005 was reached by P71 that produced 7500 g/tree. In 2006 the 3 clones surpassing 10000 g/tree were P28, P71 and P35, whit P35 showing the higher production (12900 g/tree). The other clones produced average 7860 g/tree in 2016. In 2007 the 3 clones having higher productivity in 2006 (P28, P71 and P35) and P30 produced less g of olives/tree compared to the year before. The average yield of P28, P71, P35 and P30 decreased from average 10712 g/tree in 2006 to 7475 g/tree in 2007. Average yield of the other clones (P21, P49, P18, P08 and P66)

increased from 7642 g/tree in 2006 to 10840 g/tree in 2007. P18 and P66 had the higher productivity in 2007 with 13200 g/tree and 15000 g/tree respectively.

From planting in 2002 to 2012 trees were never pruned. From 2008 to 2014 no olive productivity data were recorded. In 2012 olive tree were severely pruned and trees productivity was again assessed from 2014. From an average yield of 9344 g/tree in 2007 (going from 5800 g/tree for P30 to 15000 g/tree for P66), olive productivity recorded in 2014 fell to average 893 g/tree. Among clones, P66, with 1925 g/tree, is the clone with higher productivity in the first year of yield recording after pruning. Generally, the 3 clones surpassing 1000 g/tree in 2014 were P08, P66, P28 and P35; the other 6 clones had an average yield of 389 g/tree. In 2015, the clone which showed the highest yield was P21, increasing from 180 g/tree in 2014 to 4046 g/tree. All the other clones reached a production of average 2543 g/tree. In 2016 P08 produced 4778 g/tree, reaching the highest yield for this year; then P21 and P28 produced average 3775 g/tree and all the others between 2421 to 3106 g/tree.

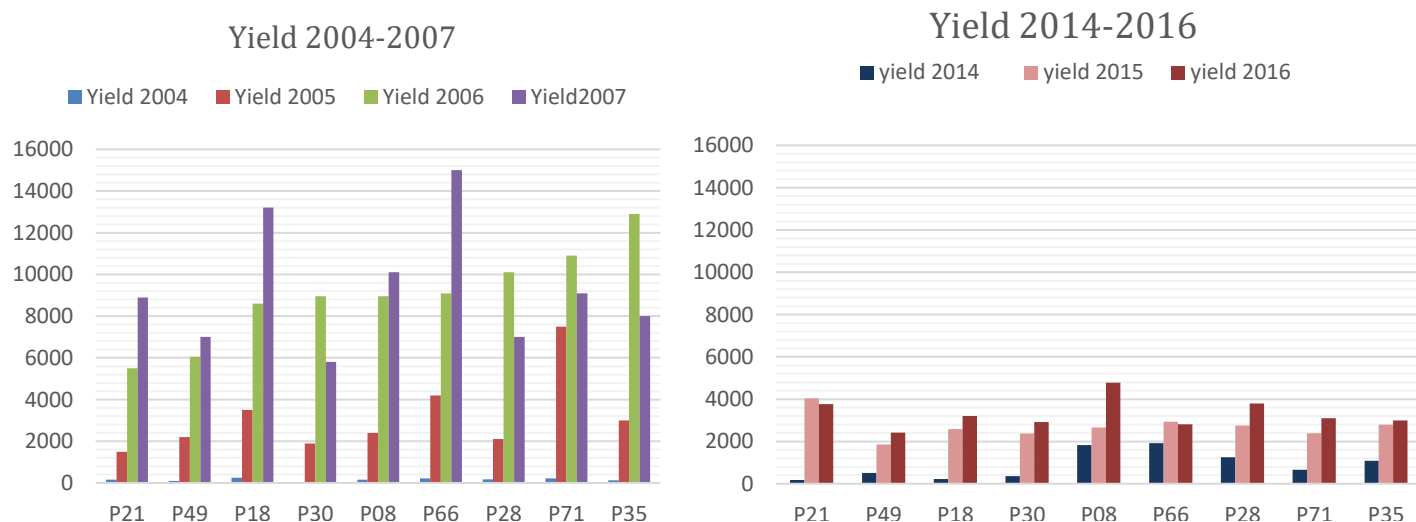


Figure 21 Annual average yield (gr/tree) of each clone from 2 years after planting to 2007 on the left (natural grass between rows) and over the 3 years of the present experiment from 2014 to 2016 on the right. Data from “ONIOL, 2007” for Table on the left.

3.4 Soil nutrient content analyses

As durum wheat was sown for the first time in 2014 we observed the evolution of NH_4^+ and NO_3^- concentration in the soil over the 3 years after the beginning of intercropping (Table 20). The higher nitrogen content was found in the first 30-cm layer for the whole period 2015-2017. A higher concentration of nitrates compared to ammonium was also shown at any depth and for the 3 years. For the 3 soil horizons, this same tendency was seen: N content decreased from

2015 to 2016 and increased from 2016 to 2017 (not always significantly and except from NO_3^- in the first horizon).

In the first 30 cm of soil profile NH_4 concentration increased in 2017 but did not reached the same concentrations it had in 2015; NO_3 concentration then decreased from 4.47 ppm in 2015 to 3,21 in 2017 (no significance has been shown). In the deeper parts of soil profile, both in horizon 2 and 3, NH_4 and NO_3^- concentrations reached in 2017 higher levels compared to 2015: significantly differences were seen for NH_4 content between 2016 and 2017.

Table 20 Ammonium and nitrate concentrations in the soil (mg/Kg TS, where TS=total solids) from AGF soil samples during the period 2014-2016 (soil samples position where in the middle of the alley between olive tree rows). Hor1 correspond to 0-30 cm depth in the soil, Hor2=30-60 cm depth in the soil, Hor3=60-90 cm depth in the soil. Means with different letters are significantly different according to Tukey's HSD

	HOR 1		HOR 2		HOR 3	
	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-
2015	1,85 (±0,6) a	4,47 (±2,9) a	0,88 (±0,4) ab	1,85 (±0,9) a	0,94 (±0,4) ab	1,77 (±0,9) a
2016	0,81 (±0,3) b	4,31 (±3,4) a	0,56 (±0,2) b	1,25 (±0,7) a	0,54 (±0,08) b	0,98 (±0,4) a
2017	1,5 (±0,8) a	3,21 (±1,2) a	1,22 (±0,5) a	2,09 (±1,8) a	1,24 (±0,6) a	1,78 (±1,3) a

We then analyzed more in details the samples positions within the olive orchard: the samples taken in the part of the orchard where durum wheat were cultivated in rotation with legume crops within the 3 years, and the samples taken in the “forest” control part. No significantly differences were seen in the N content between agroforestry system and control, at any depth and for every year. Even though the difference between the two systems got higher from 2015 to 2017. This tendency was seen mainly for NO_3^- : in 2017 3.4 ppm in agroforestry and 2.8 ppm in the forest control for the first horizon, 2.4 in agroforestry and 1 in forest control for the second horizon, 2 ppm in agroforestry and 1.2 in forest control for the third horizon.

3.5 Economical impact of agroforestry

The economic analysis is carried on to determine the potential interest of the Agroforestry system based on olive tree and durum wheat as understorey crop. We have proceeded in this evaluation by analysing profitability of the 3 systems' subparts: organic durum wheat, organic olive orchard, intercropping advantages.

3.5.1 Organic durum wheat

Table 21 Economic profitability arising from durum wheat in agroforestry system

Yield organic sole crop tonnes/ha	Yield in organic agroforestry tonnes/ha	Organic durum wheat market price euros per tonne	Gross profit euros/ha	Production costs euros/ha	Direct profit euros/ha
2,2	1,32	390 euros	514,8 euros	260 euros	255 euros
Average Yield of the 25 durum wheat varieties grown as sole crop (agronomic control) (ITAB, 2013)	40% of reduction comparing to sole crop average yield data from the present agroforestry project (harvesting 2016)	335-445 €/ton average during the period 2014/2017 revenuagricole.fr	Yield x market price	130 euros/ha for production input (seeds, fertilization) 130 euros/ha for mechanical operations threshold cost to be competitive (Arvalis 2013)	Gross profit – Production costs

To place this evaluation in the actual work conditions which farmers must cope with, we took into account the present potential yield, the production costs and the market prices within the South of France organic farming context (Table 21). The average Yield of the 25 durum wheat varieties grown as sole crop (agronomic control) was 2.2 t/ha, and it is very close to the yield of references given by ITAB (2013) for organic durum wheat crop cultivated as sole crop in the South of France. This productivity decreased when durum wheat is cultivated in agroforestry conditions between olive trees row.

The yield of the 25 durum wheat genotypes grown in agroforestry was always reduced compared to the control but the reduction range from 5% to 80%, with an average of 40%. Applying this level of reduction to the 2.2 t/ha, results in 1.3 tons/hectare. This can be considered as the potential of durum wheat yield in organic agroforestry.

The market price of organic durum wheat has been average 390 euros/tons during the past 3 years (revenuagricole.fr). Therefore, the gross profit for the farmer is about 515 euros/ha. After considering the production costs for cultivation input and mechanization of around 260 euros/ha (Table 5), we may estimate a direct profit of 255 €/ha. This estimation doesn't integrate the other crops of the rotation and the eventual need of workforce and it considers a level of yield reduction around 40%. Then, considering one hectare of olive orchard, we must consider in this calculation the presence of olive trees rows. The 6 m between the two olive tree trunks are defined by 2.2 m of ground soil covered by natural grass, where the tree row is placed, and by 3.80 m in the middle, where the wheat is cultivated. The ground soil available for the wheat in one hectare of olive orchard is then 6330 m², thus leading to a yield of 0.84 ton per hectare of olive orchard.

But if the farmer may choice a variety more adapted to agroforestry and showing only 5% of yield reduction, he may obtain a potential durum wheat yield of 2 tons/ha in agroforestry conditions. Integrating the same market price and the same production costs as in Table 4, we would reach a potential gross profit of 780 euros/hectare and a potential direct profit of 520 euros/ha. Considering one hectare of olive orchard, the yield of wheat obtained over the surface available for the crop decreases to 1.27 ton per hectare of olive orchard. .

3.5.2 Organic olive orchard

As already seen in Table 19 and 20, olive trees productivity observed in the experimentation shows to increase over the 3 years. Average yield increased from 893 g/tree in 2014 to 2710 g/tree in 2015 and then to 3309 g/tree in 2016. Due to the great difference in average yield between 2014 and 2015, we decided to consider for this economic analysis only year 2015 and 2016. For each zone, we calculated the average increasing of 2016 yield (g/tree) related to 2015 yield (g/tree). Percentages of the relative increasing are shown in Table 6. Agroforestry zones (yellow colour) showed in 2016 an average of increasing yield of 41% related to 2015. Forest zones (green colour) showed an average increasing yield of 34%. Then, olive trees in agroforestry zones increased the productivity/tree by 7 percentage units more than control from 2015 to 2016 harvesting (Table 22).

We proceeded in the economic analysis as done for organic durum wheat part. Picholine olive trees reaches in the Mediterranean south of France an average of 10 ton/ha in irrigated conditions and 4 ton/ha in non-irrigated (Afidol, 2015). Productivity of the Picholine orchard in

the study were on average 3.3 Kg/tree in 2016, thus 0,85 ton/ha considering 256 trees/ha (Afidol, 2015).

Table 22 Productivity of olive orchard zones in 2015 and 2016. In the last column, the increasing yield from 2015 to 2016 was calculated in relation to 2015 production and then presented in percentage.

olive orchard zones	initial average yield (g/tree)	inter-row	average Y/tree 2015 (g)	average Y/tree 2016 (g)	% yield variation 2016/2015
zone 1	400	crop	2872	2028	-29%
zone 2	400	crop	1723	2850	+65%
zone 3	400	crop	3425	4466	+30%
zone 4	400	grass	1585	1770	+12%
zone 5	700	crop	2163	2610	+21%
zone 6	700	crop	1813	3947	+117%
zone 7	700	grass	1692	2646	+56%

Table 23 shows the economic evaluation based on Picholine productivity within organic olive orchard in South of France. Applying the increasing of 7%, we obtain an additional yield of 0.7 ton/ha for irrigated orchard and of 0.3 ton/ha for non-irrigated orchards. These amounts allow to obtain additionally between 70 (irrigated conditions) to 30 (non-irrigated conditions) litres of olive oil (1 litre of Picholine olive oil with on average 10 kg of olives (Afidol, 2015). Considering a selling price of 5 €/L for organic olive oil, a total amount of 150 to 350 euros/ha would be obtained whole.

Lastly, by adding the profitability arising from the additional olive tree productivity and from the organic durum wheat, we can estimate the additional income from the whole agroforestry system. 255 euros/hectare is the potential profit after selling the organic durum wheat to the market. Average 250 euros is the potential additional income coming from a 7% increased olive tree productivity. Hence, adding the two subparts of the economic analysis, 505 euros/ha represent the potential global profitability of this agroforestry system.

Furthermore, if the farmer may choice a durum wheat variety adapted to agroforestry and showing only 5 % of yield reduction, the potential direct profit, arising from the cereal selling,

would reach approximately 520 euros/ha, as calculated in part 1). Integrating the 250 euros/ha from the additional olive oil sold, the potential profitability of the system would get to 770 euros/ha.

Table 23 Economic profitability arising from organic olive tree productivity in agroforestry model

Yield organic Picholine olive orchard tons/ha	Additional Yield in organic agroforestry tons/ha	Olive oil from additional productivity Litres	Organic olive oil market price euros per litre	Additional gross profit euros/hectare	Potential global profitability of the agroforestry system euros/ha
4-10	0,3-0,7	30-70	5	150-350 euros	505
Average Yield obtained in non-irrigated or irrigated conditions in the South of France territory (Afidol, 2015).	7% of increasing yield comparing to control average increasing yield related to 2015 in the present agroforestry project (harvesting 2016)	Litres produced from 300-700 Kg of Picholine olives 10 Kg of olives = 1 litre of Picholine olive oil (Afidol, 2015)		Yield x market price	Summing 250 euros: average additional profit from Agroforestry olive trees 255 euros: additional yield from intercropping durum wheat within an organic olive orchard

4. Discussion

The present study arises from the difficult economic sustainability of organic olive orchard, due mainly to low productivity. Thus, there is the need to assess in which terms implementing an agroforestry system could increase the olive orchard global profitability. Literature provides several examples of increase of olive productivity due to understorey crop. This yield's increase vary according to soil characteristics and soil management. Martinez Raya et al. (2006) showed an increase of 5–10% by maintaining a cover crop in the orchard which resulted in a better conservation of soil fertility and the avoidance of runoff losses; the role of leguminous cover crops in improving the profitability and the sustainability of rainfed olive orchards (Correia et

al., 2015) has been recently studied in the Northeast Portugal and a 53 and 95% higher cumulative yield was reached through annual legume cover crops compared to ordinary tillage techniques and natural vegetation respectively.

To date, experimentation mainly focused on assessing the impact of integrating a cover crop on the yield and then on the profitability of the olive orchard. Not much has been written on cereals as potential associated crops in agroforestry model within an olive grove. In our study, we aimed to investigate the potential role of a crop rotation based on the cereal durum wheat to improve the olive yield, the system sustainability and the orchard profitability. We observed that olive trees in the orchard zones where intercropping was implemented (agroforestry zones) increased their productivity more than the ones where the ground soil stayed covered by natural grass (forest zones). Over the three years of monitoring the increase due to intercropping was 7 % compared to the control and it determine an income of 250 euros/ha according to our analysis.

Then, the additional income coming from selling durum wheat grains depends on the adaptability of the wheat variety to agroforestry conditions. Considering a reduction of 40% compared to sole crop, which is the average percentage of decreasing in the yield of agroforestry treatments compared to control in our harvesting year 2016, the selling of this durum wheat produced would bring 255 euros/ha to the farmer. The global profitability, after adding the income arising from additional olive oil produced, would be 505 euros/ha.

If durum wheat varieties adapted to agroforestry would be improvided by breeders, they could reach higher yield when intercropped with olive trees and increase the olive orchard sustainability. A durum wheat variety decreasing its yield by only 5 % in agroforestry compared to sole crop would produce 2.09 ton/ha, considering the average 2.2 ton/ha reached in sole organic durum wheat crop in southern France. This yield would bring to the farmer 805 euros/hectar, a potential income increased by 60% compared to the one reached with wheat varieties non-adapted to agroforestry.

CONCLUSIONS AND PERSPECTIVES

This report is the result of a 6 months' internship realized in the frame of the european project AGFORWARD: AgroForestry that will Advance Rural Development. The project aims to understand the context and extent of agroforestry system in Europe, to identify and field-test innovations to improve the benefits and the viability of agroforestry systems in Europe and to evaluate innovative agroforestry designs and practices for locations where agroforestry is currently not practiced.

In 2014, in the frame of this project, the experiment object of this report was implemented. For four years, the durum wheat/olive tree intercropping system was studied. To respond to the objectives above, there is the need to investigate this farming model by considering all the aspect of its complexity. For this reason, during the 6 months I could be part of this project, numerous studies were carried on and a large set of data were collected. Moreover, the considerable number of genotypes considered and the number of treatments studied (3 in the field and 2 in the greenhouse) increased additionally the number of data, and the time for monitoring activities.

From my arrival till the end of May we carried on weekly notations both in the field and in the greenhouse: the phenological notations, SPAD and LAI recordings and measurements of plant height among them. Then, at the end of June, the plant phenotyping at maturity and after harvesting was a crucial moment of our experiment because it let us to get a lot of information of our trial results and to well characterize the varieties tested. An entire month from the end of June till the end of July was devoted to this. Directly after that, samples of grains of each variety/treatment have been milled for the quality analyses realized in August. Meanwhile, other studies were carried on: for example, more than one week was devoted to the Mycorrhizal colonization analyses in the LSTM laboratory at CIRAD Baillarguet, which results are presented in the first chapter. Moreover, we studied the respective position of durum wheat and olive tree roots in the soil profile thanks to pedological holes we dig; but there was not the time to analyze the results obtained, as those from other studies carried on, in order to present them in this report.

A great part of my internship was devoted to notations and data recordings, and I am glad of it. This gave me the possibility to achieve experience over a great variability of data types (climatic, phenological, phenotypic, pedological as examples) and protocols (Mycorrhizal coloration and SE-HPLC in particular). I believe that broaden our Knowledge over a variability of situations and scopes is necessary to achieve for a "projet de fin d'études".

Despite the time was not enough to analyze and present all data in this report, the objective, at this stage, was to show the complexity of the Agroforestry farming and amount of factor that must be considered to understand it deeply: indeed, we need to investigate its impacts on microclimate parameters if we want to understand its effect on crop phenology; then we need to investigate the effects on phenology if we want to understand the impacts on the yield crop. Moreover, we must consider a wide variability of durum wheat varieties and of genetic profiles if we want to find out which are the morphological traits of the ideotype adapted to agroforestry. Then, to promote the spread of agroforestry farming, we must provide farmers with shade – tolerant varieties. Looking for an appropriate breeding test which let to test a great number of varieties in a limited place would then be useful. Lastly, farmers sustainability need to be assured: thus evaluating the potential economical impact of Agroforestry farming was also an important issue in this study.

This was the route overall this project and overall my internship.

For this reason, the structure of this paper has been divided into 5 parts, each one presenting the results achieved according to these five topics during my internship.

For now, the aim was to show the complexity of the results obtained for each of the 5 topics.

Now, what presented in this paper will let to investigate deeply the interaction among all these factors: Climate, Phenology, Yield, Morphology, Genetic Variability. As I will be part of this project for 3 further month (CDD), my objective now is to go deeply in the analysis of data and to understand better the link between all variables studied. Moreover, I will focus on the genetic variability tested in the trial in order to better characterize each variety. This process will help to identify the traits of the durum wheat ideotype adapted to Agroforestry.

The beginning of this second step analyses will be presented during the discussion of this report.

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