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# *Cytisus* Legume Association Interests on Oak Growth and Cork Quality

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## ABSTRACT

**Background:** The cork oak is the major forest element of northern Tunisia, especially in Kroumirie. It is a species of greatecological, economic and social value, with a natural distribution limited to the western Mediterranean basin. Following serious biotic and abiotic problems, oak forests have been greatly affected and their natural regeneration has become very difficult, even almost absent these last years marked by last climatic changes. The association of cork oak with autochthonous plant species such as leguminous is an original research to resolve this problem. As part of this reflection, the association of the cork oak with *Cytisusvillosus* could positively influence its growth and productivity by providing the nutrients necessary for its survival in the face of climatic constraints.

**Methods:** The study was conducted in two sites in the Kroumirie (Ain Draham and El Feija forests). In each site two populations of the same bioclimatic stage are chosen: a population of only cork oak and a cork oak population associated with *Cytisusvillosus*. Several parameters were measured on trees at each population: morphological (height, circumference, leaf area index LMA) ecophysiological (photosynthesis, stomatal conductance, transpiration) and cork quality (porosity, humidity, quality) parameters.

**Result:** The oaks with legumes in both Ain Drahem and Feija sites showed highly significant differences compared to the other two populations without association; for morphological and ecophysiological parameters. In the same way, statistical analysis has shown that the cork oak trees in Feija population with *Cytisus* have better cork quality than other populations. We can conclude that the cork oak behaves better and have higher growth and better cork quality in the presence of legumimous species like *Cytisusvillosus*. Such results could encourage the reintroduction of this legume especially in degraded areas of cork oaks for their maintenance and preservation especially in the current climate conditions.


**Key words:** Associations, Cork oak, *Cytisusvillosus*, Growth, Oak quality.

## INTRODUCTION

Climate change is happening and several authors agree that forest species are already being affected (Buras *et al.,* 2019; Parmesan and Yohe, 2003) and will keep on being in the future (Bakkenes *et al.,* 2002; Tuiller *et al.,* 2005; Garzón *et al.,* 2008). The current climate changes appear as factors aggravating the decline of cork oak species (Buras *et al.,* 2019; Parmesan and Yohe, 2003). The cork oak (*Quercus suber* L.) is an indigenous species of the Tunisian forest; it plays an important ecological and socio-economic role. Due to serious biotic and abiotic problems, oak forests have been severely weakened and their natural regeneration has become very random, if not almost absent. This situation has become worse because of the lack of natural regeneration and the technical operations as protection and renewal. Despite the increase of $CO_2$ atmospheric, Cork oak forests don't stop degenerating and apparently not benefit from this rise. Deficits in water balance and in nutritional elements are certainly the cause (Buras *et al.,* 2019; Parmesan *et al.,* 2003; Tuiller *et al.,* 2005). Elevated atmospheric $CO_2$ can substantially alter plant chemistry and leaf surface properties. These, in turn, can alter host/pest interactions. For example, it is well documented that levels of foliar N decline for trees growing under elevated atmospheric $CO_2$ (Nabuurs *et al.,* 2000). Legume tree species may be a critical regulator of soil nutrient dynamics because of their high foliar nitrogen (N) and potential for symbiotic N fixation (Comandini *et al.,* 2006). Legumes (Fabaceae, Leguminosae) such *Cytisus* are a good test case for individual species effects on forest as they are potential regulators of ecosystem nutrient dynamics (Fisher, 1995; Franco and De Faria*,* 1997; Wang *et al.,* 2010). They are the most diverse and widespread group of plants with the capacity of $N_2$ fixation (Sprent, 1995; Sprent and Parsons, 2000). In the north west of Tunisia, known as "hedhben",

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*Cytisus villosus* Pourr is the spontaneous species which growing naturally throughout the Kroumirie.

Understanding the impact of these legume species on forest trees (cork oak), in particular assessing potential benefits from increased legume-nitrogen-fixation, can provide important information for future land-management and policy decisions.

## MATERIALS AND METHODS

A morphological and ecophysiological study of cork oak trees and their cork quality (porosity, humidity, durability) was carried out in four cork oak populations in Feija and Ain Drahem areas in Jendoubagouvernorate (AD1 with Cytisus; AD2 without Cytisus; Fey1 Cork oak with *C. villosus* and Fey2 without Cytisus) (Table 1).

### Morphological parameters

Morphological parameters were measured on all oak trees in each population: height (m) and diameter (cm) at 1.30 m from the base of the trunk. Then, a sampling of Cork oak leaves was performed in August, on the seven representative trees within each population. Mature leaves were collected randomly on each sampling. The foliar surface masses are obtained by estimation of the average surface of the leaves and measure of the dry weight (The total projected leaf areas were determined by an image analysis system (Delta-T Image Analysis System, Delta-T Devices Ltd., Great Britain):

$$\text{LMA (g/m}^2\text{)} = \frac{\text{Dry mass (g)}}{\text{Foliar surface (m}^2\text{)}}$$

### Gas exchanges

Gas exchanges were measured using a Li-Cor Li-6400XT Portable Photosynthesis System (Li-Cor, Lincoln, NE, USA) based on the IRGA principle (Infra RedGas Analysis). The leaf stomatal conductance (g, in mol $H_2O$ $m^{-2}s^{-1}$), net carbon assimilation (A, in µmol $CO_2$ $m^{-2}s^{-1}$) and transpiration (T, in mmol $H_2O$ $m^{-2}s^{-1}$), were measured on the expended leaves of cork oak. More than eight branches were taken from each site. Under appropriate conditions, they were cut and transported to the laboratory for measurements.

### For cork parameters

6 representative trees of each population, in perfect health and having reached a potentially exploitable age (9 years) were selected. From these trees, the cork was removed (a 20 × 20 cm area from 1.30 m the ground).

In the laboratory, after a week, the samples were processed (boiling, sorting and stabilization). Cork plugs were then manufactured and parameters measured:

$$\text{Density} = \frac{M}{V}$$

Where,

V= L1*L2*E

M: Mass (weight); V: Volume; L1: Length 1; L2: Length 2; E: Thickness; V= L1*L2

We measured the density of planks and corks after boiling for each population.

Porosity= Weight before boiling - Weight after boiling

Humidity with the electronic hygrometric method, using the "aqua-boy" device with electrodes inserted into the sample.

**Quality:** Classification criteria are based on five thickness classes and 7 appearance classes.

**Tightness (corking test)**

**Dimensional control:** The acceptance criteria are plugs with Length ± 0.5 mm and Diameter ± 0.4 mm.

**Waxing test:** By immersing corks in mineral water. Samples of corks will be accepted if they are TCA (trichloroanisole) free.

**Sensorial test:** Sensorial test measured after cap has been immersed in 50 ml mineral water for 24 hours.

## RESULTS AND DISCUSSION

### Morphological and ecophysiological parameters

Statistical analysis of morphological and structural parameters showed highly significant differences between sites. The oaks at AD1 differed from their fellows (AD2, Fey1 and Fey2) by having the highest values for diameter (39 cm±5.8 cm) and height (10.61 m±2.7 m) and the lowest values for LMA (107.80 g/m²±14.91 g/m²) (Fig 1). However, trees on AD2 (on the north versant, without legumes) and Fey1 (on the south versant, with legumes) showed no significant differences in these parameters (Fig 1).

This study aims to elucidate the impact of legume association on growth and productivity of Cork oak (*Quercus suber* L.). Legumes (Fabaceae, Leguminosae) are a good test case for individual species effects on Mediterranean soils as they are potential regulators of ecosystem nutrient (Franco and De Faria, 1997; Wang *et al.,* 2010). Results suggest that the cork oak behaves better in the presence of legume (best growth in height and diameter for AD1 with

**Table 1:** Site characteristics.

| Provenances | Populations | Stage | Altitude (m) | Lambert coordinates | | Average temperature | Annual rainfall (mm/year) |
|---|---|---|---|---|---|---|---|
| Ain Drahem (AD) | -AD1 (with Cytisus) | Humid | 800 | 36°47 26.89N | 8°41 16.22E | The coldest: 6.6°C | 1500 |
| | -AD2 (without Cytisus) | | 665 | 36° 47 41.07N | 8°40 59.79E | The warmest: 24.9°C | |
| Feija (F) | -Fey1 (with Cytisus) | Sub- | 780 | 36° 29 4.82N | 8°18 4.84E | The coldest: 9.93°C | 1200-1500 |
| | -Fey2 (without Cytisus) | humid | 760 | 36° 29 40.14N | 8°18 18.15E | The warmest: 28.7°C | |

the lowest LMA values). The *C. villosus* is beneficial for the oak growth. In fact, the root system of the oak is pivotal while that of Cytisus is superficial. Indeed, the two species are not in competition for water or nutriments. A deep and dense root development allows the tree to permanently balance water losses due to transpiration (Palliotti *et al.,* 2000).

For the eco-physiological parameters, results show significant differences in photosynthesis, stomatal conductance and transpiration between sites with and without legumes. AD1 and Fey1 (with legume) had the highest photosynthesis and conductance values and the lowest leaf transpiration values (Fig 2).

Under harder climatic conditions such as lower water availabilities and high temperatures; Cork oak in Fey1 tends to have more leaves with higher LMA, which is more efficient to maximize photosynthetic gain and invest more resources in strong and rigid leaves to resist climatic adversities (Ogaya *et al.,* 2011). With legume, the trees transpire less than those without Cytise (Fig 2). High LMA values in Mediterranean vegetation are often related to leaf resistance to dry conditions (Niinemets, 2001) and to high vapor pressure deficit and potential evapotranspiration (Wright *et al.,* 2004). The greater LMA normally shown by the leaves at the drier sites have been interpreted as a mechanism that allows leaves to increase their resistance to drought and improve their water use efficiency (Turner, 1994; Niinemets, 2001; Wright *et al.,* 2001). *C. villosus* is a legume that presents intermediate characteristics between Sclerophyllous spp. It is considered as stresstolerating and summer deciduous species (Mooney and Kummerow, 1971). In fact, this species supplies the oak with required nitrogen to enhance its

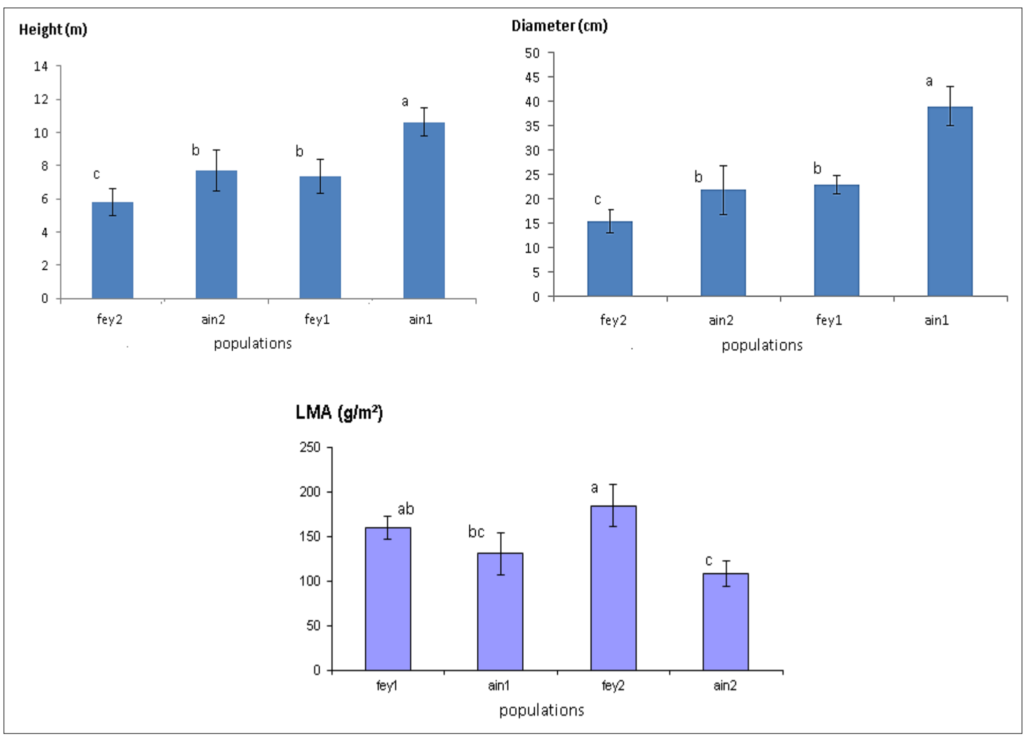



**Fig 1:** Mean (± SE) stand-level estimates of height, diameters at breast and LMA of cork oak in sites.

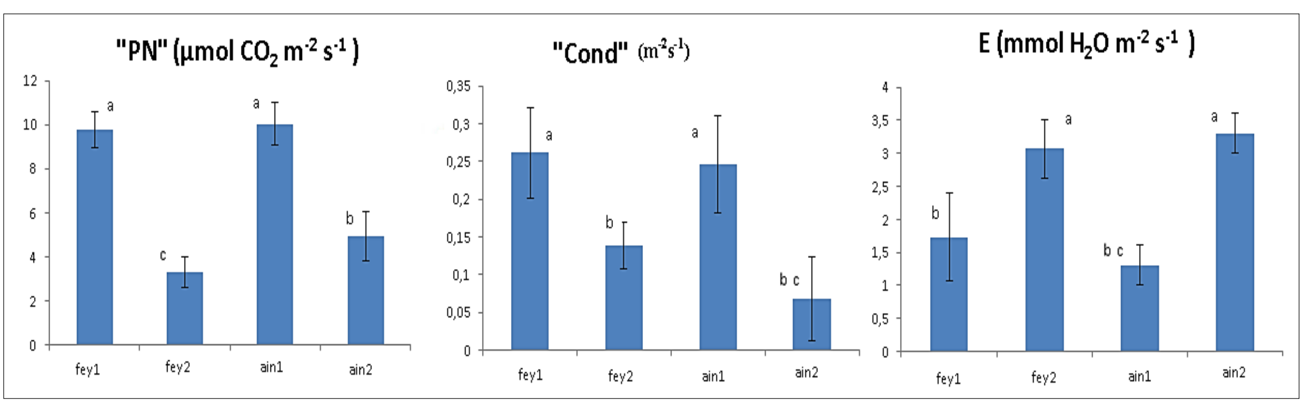



**Fig 2:** Net photosynthesis (PN), stomatal conductance (Cond) and transpiration (E) in cork oak at four sites; measuring season: August.

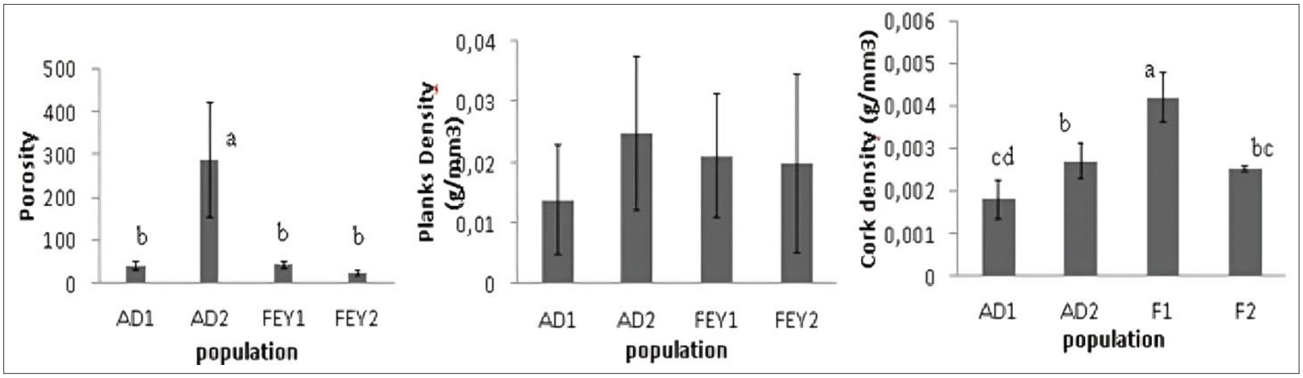
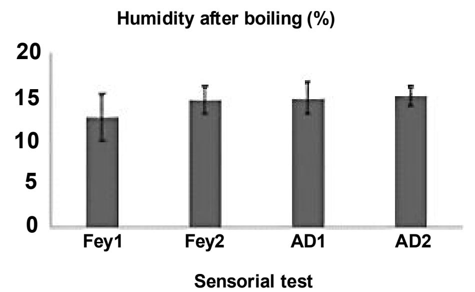



**Fig 3:** Mean (± SE) stand-level estimates of porosity and density planks and corks density.

| Corks | Sealing |
|---|---|
| F1A4B1 | Good |
| F1A4B5 | Good |
| F1A4B6 | Good |
| vF2A5B3 | Bad/Flow |
| F2A5B4 | Good |
| F2A2B4 | Bad/Flow |
| AD1A3B1 | Good |
| AD1A3B2 | Good |
| AD1A3B3 | Good |
| AD2A1B2 | Bad/Flow |
| AD2A1B4 | Good |
| AD2A1B5 | Good |

| Cork plug | Odor | Assessment |
|---|---|---|
| F1A6B6 | No odor | + |
| F2A2B1 | Dusty odor | - |
| AD1A3B5 | Vegetable odor | + |
| AD2A2B4 | Soil odor | - |




F1: Feyja1; F2: Feyja2; AD1: Ain Drahem1; AD2: Ain Drahem2; A: tree; B: cork. F: Feija; AD: Ain Drahem; A: tree; B: Cork plug.

**Fig 4:** Cork quality parameters.

tolerance to water stress. Nitrogen affects gas exchange and the photosynthetic capacity of the cork oak trees (Sprent, 1995; Sprent and Parsons, 2000). Nitrogen fertilizer with legume could promote photosynthetic performance of *Quercus suber* by stimulating morphological and physiological responses (Palm, 1995; Kachout *et al.,* 2017; Marney and Borden, 2019).

**Cork parameters**

Mediterranean region experience high water stress conditions and reduced vegetation production (Lionello *et al.,* 2017 ; Giorgi and Lionello, 2008). The cork oak (*Quercus suber*) is an evergreen oak species distributed along the western Mediterranean basin, covering an area of approximately 2.1 million ha (Oliveira and Pereira, 2019). Its outer bark (cork) is periodically (usually every 9 years) removed on a sustainable procedure, corresponding to an annual production of up to 200 thousand tons (Aroso *et al.,* 2017). This forest product is the second most important non wood forest product commercially exploited and it is the raw material of an important industry with diversifed products and applications. Likewise, the cork oak (*Quercus suber* L.) forest is a unique production system that has a substantial ecological role, against desertification and in maintaining animal and plant biodiversity in their restricted area of occurrence in the Mediterranean countries (Oliveira and Pereira, 2019). Following measurements of cork quality parameters, the Fey1 site (cork oak with legume, south versant) showed the lowest porosity (52.16±14.5) with highest density in its planks and the best density in its corks (0.004 ±g/mm$^3$ ± 0.002 g/mm$^3$) (Fig 3). The cork quality measurements revealed that corks from populations with legumes (Fey1 and AD1) have a very good quality. In fact, corks meet the sealing requirements of wine bottles (Pereira, 2015; Oliveira and Pereira, 2019) (Fig 4) and the sensorial test revealed that Fey1 corks haven'todor and AD1 corks have a very good vegetal odor (Fig 4).

The entire cork chain from the forest to the consumer relies on the regular and sustainable production of cork. To maintain cork production capacity and provide the mentioned environmental services, it is necessary that cork oak forests are adequately managed, being the sustainability a matter of general concern (Oliveira and Pereira, 2019; Pereira, 2007). Our cork study revealed that the best quality is found in the cork oak trees with Cytisus at Fey1, (Fig 3 and 4). High-density cork gives good cork quality oaks are found in association with *C.villosus* (in Fey1 and AD1). Fey1 and AD1 are two populations from two different versants and micro-climate. What unites them is essentially their association with *C.villosus*, which, as our results show, is beneficial to their growth and cork quality.

## CONCLUSION

Our research confirms that *Cytisusvillosus* proved beneficial to cork oak growth and cork quality. Such results could encourage the reintroduction of this legume, especially in degraded areas of cork oak, to maintain and preserve them, especially under current climatic conditions. This association can play a very important role for the cork oak with regard to the mineral elements existing in the soil. This study clearly demonstrates the importance of preserving this type of forest heritage. Oaks planted in association with Cytise are healthier and therefore of greater economic, social and environmental value to both the local population and the state. It's not just a question of preserving an interesting ecosystem, but also an original form of silvicultural management.

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**Conflicts of interest**

The authors have no conflicts of interest to declare. All co-authors have seen and agree with the contents of the manuscript and there is no financial interest to report. We certify that the submission is original work and is not under review at any other publication.

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