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Effect of *Pinus halepensis Mill*. Reforestation on the Above-Ground Biomass and Internode Elongation and Leaf Size of Native Species in Morroco

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Abstract

This study aims to determine the effect of the reforestation of *Pinus halepensis Mill.* on the above-ground biomass and morphological characteristics of native species (internode elongation and leaf size) in the Beni Sohane forest. Plant biomass of the herbaceous layer was harvested on randomly selected 2 m^2 quadrats in reforested plots of ± 12, ± 25, and ± 45-year-old, and native forest controls. The internode length and leaf size (length and width) were measured on plants randomly selected belonging to four native species *Quercus ilex L., Pistacia lentiscus L., Phillyrea angustifolia L.*, and *Cistus creticus L.*. The results showed that *P. halepensis* reforestation had no significant effect on the above-ground biomass and the leaves and internodes dimensions in the young plantations ± 12-year-old. However, as the pine trees mature, the average dry matter weight decreases, especially in stands 45 years old, where this weight was significantly lower than that of natural forests. This fact has led to herders abandoning important pastures previously used by their cattle. In addition, the fast growth of *P. halepensis* trees formed a canopy above all indigenous species resulting in changes in the dimensions of internodes and leaves. For the two oldest plantations, the internode length, leaf width, and length of the 4 species have been significantly increased.

However, the leaf length-to-width ratio decreased significantly, with leaves in reforested plots being larger rather than longer compared to control samples in native forests. Planting *P. halepensis* can negatively impact the long-term growth of native plants, so we recommend periodically removing some of the pines (thinning) to restore the balance of these ecosystems. Thus, species selection for reforestation should consider maximizing rather than destroying ecological and socioeconomic services.

Keywords: Reforestation; Pinus halepensis; Phytomass; Exotic species; Shade

1. Introduction

Mediterranean forests have been affected by human activity for centuries. This effect became more pronounced towards the end of the last century as the population and its needs increased. As temperatures rise and precipitation decreases, the composition and overall growth of forests may change dramatically. As a result, some tree species that were once prevalent in an area are dying out, with these native species being replaced by more tolerant invasive species (Lawson & Michler, 2014). Afforestation and reforestation are among the most used strategies to mitigate the effects of climate change, given that, trees capture carbon dioxide (CO_2) from the atmosphere as they grow and store the carbon in living biomass.

Since the end of the last century, the Beni Sohani Forest has experienced severe degradation due to intense anthropogenic pressure. To remedy this situation, the Water and Forestry departments have reforested large areas, using Aleppo pine (*Pinus halepensis Mill.*) as the main tree species. Reforestation included degraded areas and many areas containing native tree species, e.g. (*Quercus ilex L., Juniperus oxycedrus L., Juniperus phoenicea L., Tetraclinis articulta (*Vahl*) Mast., etc.)*

Since the 1970s, *P. halepensis* has been one of the most used Pinus species in reforestation in the Mediterranean Basin and in many other regions outside its native range (Bello-Rodríguez et al., 2020). It has several properties that make it ideal for reforestation and afforestation projects, such as protection of degraded areas, drought tolerance, fire resistance, rapid growth, soil adaptability, erosion control, and timber production (Bello-Rodríguez 2020 et al. ; Mechergui et al., 2022).

Over the past one million years, human activities have radically altered the natural distribution of the pine, blurring the boundaries between the natural and introduced distribution areas of all native pine species in the Mediterranean basin (Richardson & Nsikani, 2021). Moreover, *P. halepensis* is now considered an invasive species in different parts of the world (Mechergui et al., 2022). We considered this species an invasive species because it was only introduced in the 1970s to our study area, which was primarily covered with green oaks.

In Beni Sohane Forest, our previous studies showed that the reforestation of *P. halepensis* has negatively affected richness, and floristic diversity (Benarchid et al., 2018), and soil fertility (Benarchid et al., 2022). Several studies report a lack of recovery of spontaneous vegetation in *P. halepensis* plantations 23 years after planting in SE Spain than those reported in adjacent shrublands not reforested (Chaparro & Esteve, 1996). The transformation from shrub-dominated to tree-dominated landscapes of *P. halepensis* in a semi-arid area reduced the productivity of the herbaceous species (Paz-Kagan et al., 2016). On the other hand, the canopy formed by *P. halepensis* trees may affect the morphological characteristics of native species. Changes in light gradients in the canopy of different species and forest types produce changes in leaf traits, and morphological and biochemical adaptations to outperform competitors (Lichtenthaler et al., 2007; Ugarte et al., 2010). To explore the effects of *P halepensis* reforestation on plant biomass and native species, we measured aboveground dry matter biomass, internode length, and leaf size of four native shrubs and trees *Quercus ilex L., Pistacia lentiscus L., Phillyrea angustifolia L.,* and *Cistus creticus L.*

2. Material and methods

The study was conducted in the Beni Sohane Forest, which is located in the northern Middle Atlas. It is bounded by geographic coordinates of 584 Km and 620 Km in the west and 334 Km and 370 Km in the north (Figure 1). The altitude is between 800 and 1200 meters. The average annual rainfall is 550 mm. Emberger's climagram categorizes the study area as being in the subhumid bioclimatic stage in cool winter (Emberger, 1955). The average temperature of the hottest month is 35.46°C, while the average temperature of the coldest month is 2.45°C. The main soil types are poorly evolved, raw mineral, calcimagnetic, isohumic, and fersiallitic (S.E.I, 2014).

The Beni Sohane Forest experienced three waves of reforestation. The first one began in the 1970s (i.e. trees around 45 years old). The second wave occurred about twenty years later (i.e. trees around 25 years old), and the last one happened during the first decade of the 2000s (i.e. trees around 12 years old).

The observations were carried out on the plant biomass of the herbaceous layer in reforested plots of ± 12, ± 25, and ± 45 -45-year-old, and native forest controls. Above-ground plant material on the ground has been cut using a semi-destructive method on randomly selected 2-square-meter plots, repeated 20 times. dry matter has been Determined after drying to constant weight in an oven at 60 °C (Floret & Pontanier, 1982).

The internode length and leaves dimensions (length and width) were measured on plants randomly selected belonging to four native species *Quercus ilex L., Pistacia lentiscus L., Phyllyrea augustifolia L., and Cistus creticus L*. The internodes and the leaf's dimensions (length and width) were measured at a height of between 1 and 2 meters with 40 repetitions. We measured the length along the central veins and the maximum width perpendicular to the central veins. The harvest of plant biomass has been carried out during May, corresponding to the full development of the herbaceous layer.

The statistical processing of the data concerned the comparison of the dry weight of the above-ground biomass and the leaves and internodes dimensions at the level of the reforested area and the native forest. We used Dunnett's 1-way ANOVA test after checking that the normality and homoscedasticity of the variables by the Shapiro-Wilk and Levene tests were not satisfied even after their logarithmic transformation. IBM SPSS Statistics 22 was used to conduct the statistical analyses.

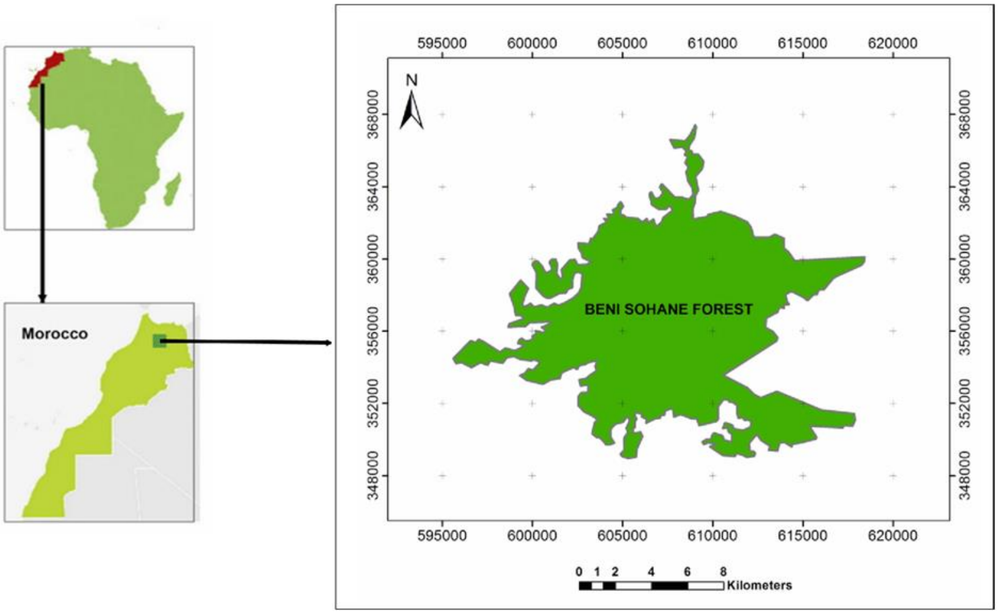

Figure 1: Location of study area

3. Results:

3.1. Effect of *Pinus halepensis* reforestation on above-ground biomass:

The measures of the weight of dry matter above-ground biomass of the herbaceous strata showed that the weight of control was higher than the reforested plots, especially for the oldest reforested plots. This weight decreased at 258g and 129g respectively in reforested plots of ± 25, and ± 45 -year-old. The statistical analysis showed that these weights were significantly lower than those recorded in the native forest (509 g). However, biomass weight was almost the same in the youngest plots of ± 12 years compared to the control samples of the native forest 486 g and 509 g respectively.

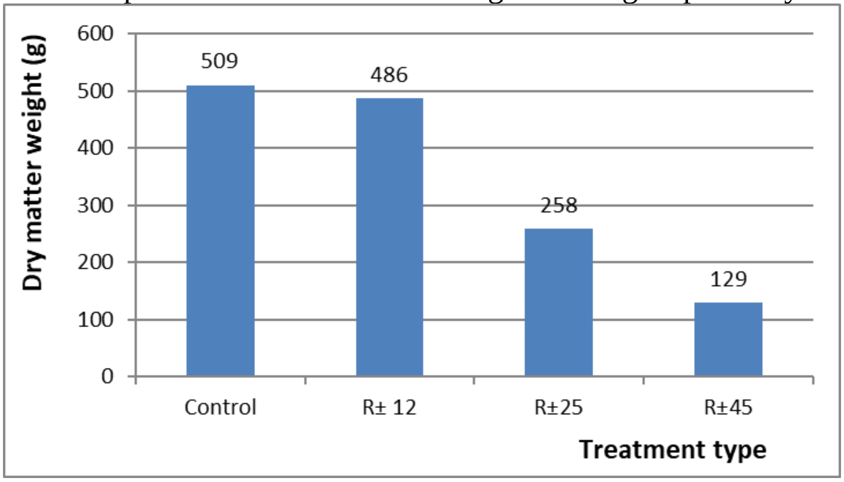

Figure 2: Effect of *Pinus halepensis* reforestation on above-ground biomass

Table 1: Dunnet non-parametric test of dry weight at 5 % significance

Designations	Mean dry weight (g)	**Signification 5 %**
	509	
R$_{\pm 12}$	486	0.993
R$_{\pm 25}$	258	0.203
R$_{\pm 45}$	129	(,0190)*

(*) : Significant difference on above-ground biomass between reforested plots and native forests at the significance level p = 0.05%.

3.2. Impact of *Pinus halepensis* reforestation on internode elongation and leaf size of native species:

Reforestation of *P. halepensis* leads to internodal elongation, which increases with reforestation age. The average internode length of reforested plots was higher than those recorded in the control samples of the native forest by 27 % for the youngest plots of ± 12 -year-old, 101% for the reforested plots of ± 25-year-old, and 167 % for the reforested plots of ± 45 -year-old. However, statistical tests of ANOVA revealed no significant difference between the youngest reforested plots and the native plots. However, the internode length in the reforestation plots of ±25 and ±45 -year-old was significantly longer than the native plots. This elongation was more accentuated for *Phillyrea augustifolia*, the average internodes length of reforested plots of the three ages was higher than those recorded in the control samples by 157 %, and 120 % for *Cistus creticus*, and 78 % for *Quercus ilex*. *Pistacia lentiscus*, on the other hand, had the lowest elongation of 39%.

Reforestation of *P. halepensis* has resulted in wider leaves, especially on older plots. The average leaf width of reforested plots was higher than those recorded in the control samples of the native forest by 24% for the reforested plots of ± 25-year-old, and 45% for the reforested plots of ± 45-year-old. However, it did not affect leaf width in ±12-year-old reforested plots.

Statistical analysis showed that only in the oldest reforested plots of ± 45 -year-old, the leaf widths of *Quercus ilex, Cistus creticus,* and *Pistacia lentiscus* were significantly larger than those of native plots. There were no significant differences in other reforested plots (Table 2). A great increase in leaf width has been recorded for *Quercus ilex* (45%), followed by *Cistus creticus* (21%), *Pistacia lentiscus* (19%), and only 0.08% for *Phillyrea augustifolia*.

Reforestation of *P. halepensis* has resulted in a slight increase in leaf length on older reforested plots. On average, leaves of reforested plots were longer than those of the control samples of the native forest by 5% for the reforested plots of ± 25 -year-old, and 10% for the reforested plots of ± 45 -year-old. However, it did not affect leaf length in ±12-year-old reforested plots. Likewise, the increase in leaf length changed little among the four species, -11% for *Cistus creticus*, 4% *Pistacia lentiscus*, 10% for *Phillyrea augustifolia,* and 16% for *Quercus ilex.*

Statistical analysis showed that in the ± 45-year-old reforestation plots the leaves of *Quercus ilex*, *Cistus creticus*, and *Phillyrea augustifolia* were significantly longer than those of native plots, while there was no significant difference for *Pistacia lentiscus*. In the ± 25-year-old reforested plots, no significant differences were found for the other species, except for *Cistus creticus*. Similarly, in the ± 12-year-old reforested plots, no significant differences were found for all species (Table 2).

Reforestation of *Pinus halepensis* has resulted in a slight decrease in the leaf length-to-width ratio on older reforested plots. On average, leaves of reforested plots became larger than longer in comparison with those of the control samples of the native forest by -13% for the reforested plots of ± 25 -year-old, and -21% for the reforested plots of ± 45 -year-old. However, in the youngest reforested plots, this proportion was not affected. Excepting for *Phyllyrea augustifolia*, this ratio decreased for *Cistus creticus, Quercus ilex, and Pistacia lentiscus* by 7%, -11%, - 25 %, and - 17% respectively.

Statistical analysis showed that in the ± 45 -year-old reforestation plots the ratio L/W of *Quercus ilex*, *Pistacia lentiscus* and *Cistus creticus* were significantly lower than those of native plots, while there was no significant difference for *Phyllyrea augustifolia*. In the reforested plots of ± 25 -year-old, excepting *Cistus creticus*, there was no significant difference for the other species. Similarly, there were no significant differences among all tree species in the ± 25-year-old reforested plots. Similarly, in the reforested plots of ± 12 -year-old, there was no significant difference for all species.

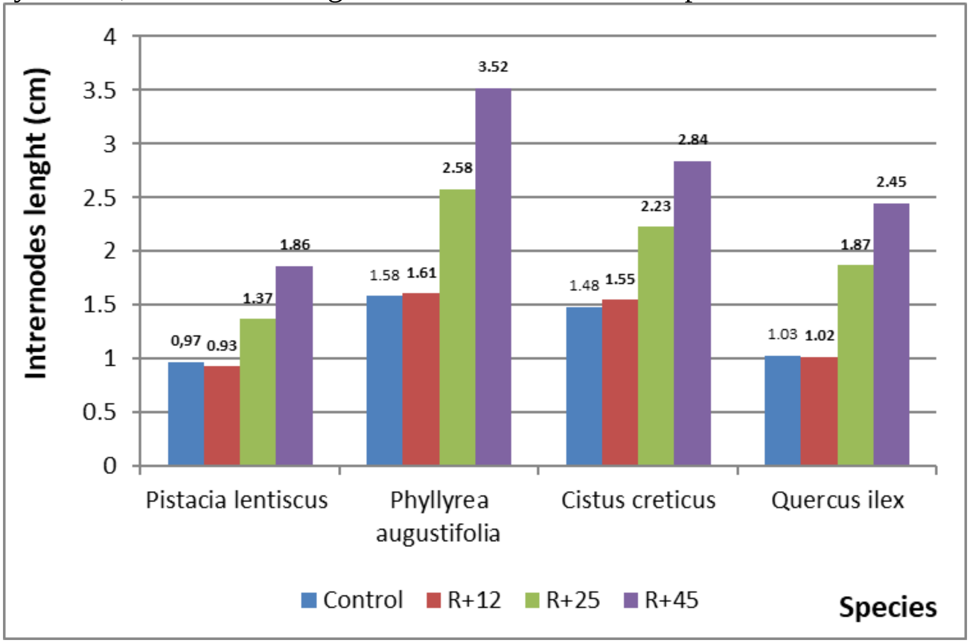

Figure 3: Effect of *Pinus halepensis* reforestation on internode length

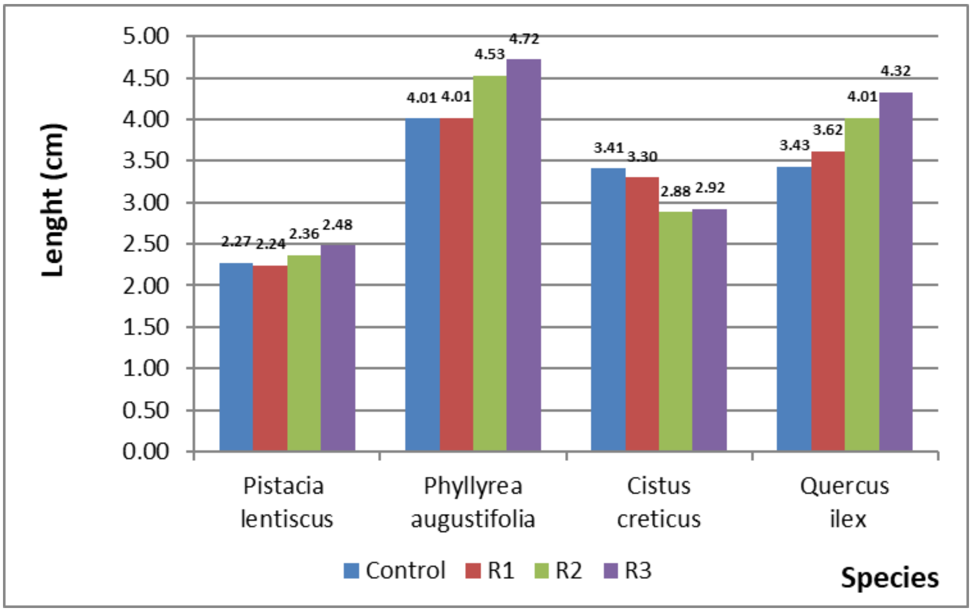

Figure 4: Effect of Pinus halepensis reforestation on leaves length

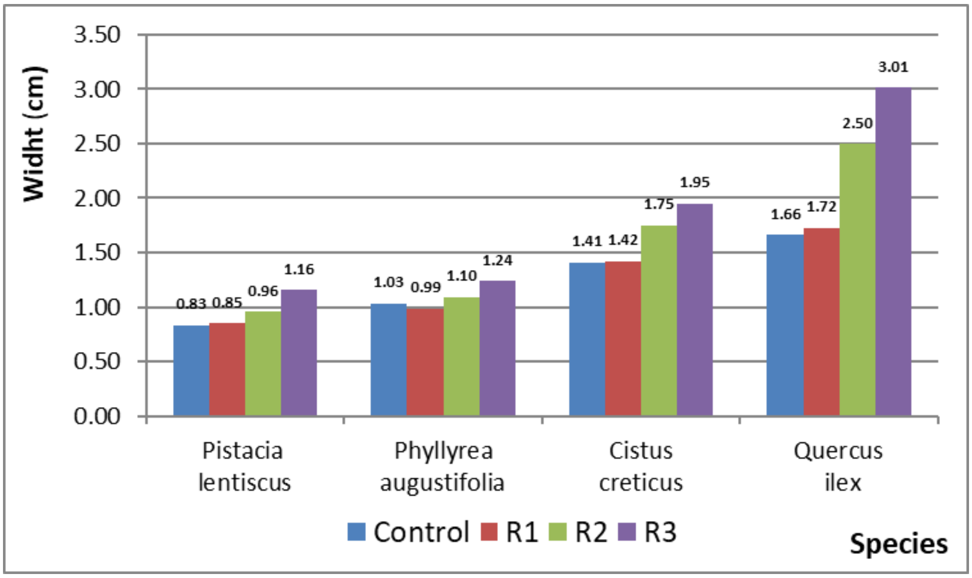

Figure 5: Effect of *Pinus_halepensis_*reforestation on width leaves

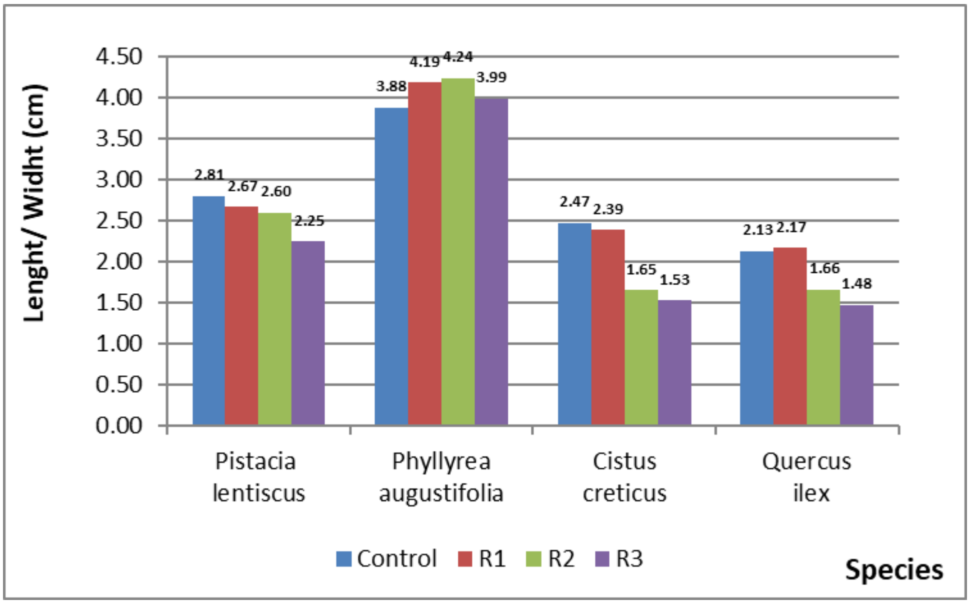

Figure 6: Effect of *Pinus halepensis_*reforestation on the leaf length-to-width ratio

Table 2 : Data in columns are means (n=40); data in brackets are Test ANOVA at 5% significance Tukey and Dunett

Designations		**Internode lenght (cm)**	**Leaves widht (cm)**	**Leaves lenght (cm)**	**Ratio lenght/widht**
Pistacia lentiscus	**Control**	0,97	0,83	2,27	2,81
	R$_{\pm 12}$	0,93 (0,979)	0,85 (0,956)	2,24 (0,994)	2,67 (0,737)
	R$_{\pm 25}$	1,37 **(0,001)***	0,96 (0,067)	2,36 (0,823)	2,60 (0,410)
	R$_{\pm 45}$	1,86 **(0,000)***	1,16 **(0,000)***	2,48 (0,208)	2,25 **(0,000)***
Phyllyrea augustifolia	**Control**	1,58	1,03	4,01	3,88
	R$_{\pm 12}$	1,61 (0.999)	0,99 (0.990)	4,01 (0.995)	4,19 (0.997)
	R$_{\pm 25}$	2,58 **(0.000)***	1,10 (0.994)	4,53 (0.016)	4,24 (0.006)
	R$_{\pm 45}$	3,52 **(0.000)***	1,24 (0.214)	4,72 **(0.000)***	3,99 **(0.000)***
Cistus creticus	**Control**	1,48	1,41	3,41	2,47
	R$_{\pm 12}$	1,55 (0.904)	1,42 (1.000)	3,30 (1.000)	2,39 (0.965)
	R$_{\pm 25}$	2,23 **(0.000)***	1,75 (0.995)	2,88 **(0.000)***	1,65 **(0.000)***
	R$_{\pm 45}$	2,84 **(0.000)***	1,95 (0.577)	2,92 **(0.000)***	1,53 **(0.000)***
Quercus ilex	**Control**	1,03	1,66	3,43	2,13
	R$_{\pm 12}$	1,02 (0.999)	1,72 (0.998)	3,62 (1.000)	2,17 (1.000)
	R$_{\pm 25}$	1,87 **(0.000)***	2,50 (1.000)	4,01 (0.020)	1,66 (0.016)
	R$_{\pm 45}$	2,45 **(0.000)***	3,01 **(0.001)***	4,32 **(0.000)***	1,48 **(0.000)***

(*) : Significant difference between reforested plots and native forests at the significance level p = 0.05%

4. Discussion

Our results showed a significant decrease in the weight of dry matter above-ground biomass of the herbaceous strata in reforested plots of ± 25-year-old and ± 45-year-old (P < 0.001) compared with the native forest. The decrease in above-ground biomass can be explained by the fact that the *Pinus halepensis* colonized all reforested plots by forming a canopy overhanging tree native species *Quercus ilex L.*, *Juniperus oxycedrus*, and *Juniperus phoenicea*, and dominating also all other species. *P. halepensis* is characterised by its great plasticity, high drought tolerance, fire adaptation, fast growth, and soil adaptability (Bello-Rodríguez et al., 2020). Its regeneration capacity is notable, and it is now considered an invasive species in different areas of the world (Mechergui et al., 2022). Several studies report a lack of recovery of spontaneous vegetation in *P. halepensis* plantations 23 years after planting in SE Spain, as well as values of plant cover in seven plantations that were, on average, 20% lower than those reported in adjacent shrublands not reforested (Chaparro & Esteve, 1996). The transformation from shrub-dominated to tree-dominated landscapes of *P. halepensis* in a semiarid area reduced both the productivity of the herbaceous species (Aboveground net primary productivity) and the soil fertility beneath the canopies of the trees, as compared to the productivity and soil fertility under the shrub canopy in a native shrubland ecosystem (Paz-Kagan et al., 2016). In shade-avoiding plants, a series of morphological adaptations help to outperform competitors and increase fitness in competitive environments, but at the expense of biomass production (Ugarte et al., 2010). The leaf characteristics of the plant also change due to shading, resulting in thinner leaves and a lower photosynthetic rate than in full sun conditions. (Terashima et al., 2006), and this is mainly due to the greater palisade parenchyma, spongy parenchyma, and epidermal tissue thickness, suggesting that leaf internal structure may play an important role in light capture (Gratani, 2014). In addition, there were fewer shrubs in the undergrowth of conifers compared to hardwood species (Rizvi, 2012). Similarly, understorey natural vegetation in P. halepensis plantations in the Green Barrage of Algeria was characterized by a considerably low cover (Benabdeli, 1998). *P. halepensis* could impact other species through the production of secondary metabolites or allelochemicals (terpenes and phenols) that can strongly inhibit the germination of other plant species (Fernandez et al., 2013). On the other hand, afforestation by *P. halepensis* had a negative impact on soil moisture, an impact that increased with tree density (Bellot et al., 2004). At the landscape scale, allelopathy in planted forests could interrupt vegetation landscape connectivity by impeding the dispersal and spread of native plant species through afforestation patches. This fragmentation of natural habitat patches could, in turn, restrict the gene flow of native species (Honnay et al., 2002).

The shade produced by the *P. halepensis* canopy also helps inhibit the development of herbaceous understory plants. In addition to competition for water and nutrients, competition for light also leads to the loss of shade-intolerant native species. Indeed, our previous studies showed that the floristic diversity, species, genus, and family richness recorded in the *P. halepensis* reforested plots were significantly lower than those recorded in the control plots. This negative impact was more pronounced for the older plantation. (Benarchid et al., 2018). Other studies in semiarid Mediterranean areas showed that extensive pine plantations reduce species richness and plant diversity in the understory (Chirino et al., 2006). Furthermore, shade treated soybean plants showed significantly decrease in stem diameters and shoot biomass and a significant reduction in cell number and cell size in leaves (Wu et al., 2017).

Nevertheless, reforestation with *P. halepensis* has been the most used method to restore degraded lands in semi-arid areas of the Mediterranean, with the main goal of improving soil conditions to induce succession (Ruiz-Navarro et al., 2009). A canopy of pines or other species can also promote facilitation processes to improve microclimatic conditions and nutrient availability and facilitate the establishment of seedlings (Maestre et al., 2003). In a variety of ecosystems where plants face water stress, shading often enhances plant growth and survival, but this is not always the case in water-limited ecosystems (Pérez-Devesa et al., 2008). In some cases, the planting of exotic species can be a catalyst for the regeneration of native species. In south-central Madagascar, affected by various disturbances (hurricanes, logging, farming), *Pinus patula* plantations have become an environment conducive to the regeneration of native species (Randriambanona et al., 2019). Improved soil conditions and shading can promote the establishment of grasses under the pine canopy (Bautista, 1999). However, despite the positive impact of *P. halepensis* plantations on the development of herbaceous understory under semi-arid conditions, overall plant cover and species richness decreased (Bautista & Vallejo, 2002). In the mesic climate region of northeastern Catalonia, Spain, oak seedlings are positively correlated with adult pine trees (Lookingbill & Zavala, 2000). However, many studies have suggested that improved microclimatic conditions by the development of *P. halepensis* canopy cannot balance the reduction in soil water availability promoted by the introduction of *P. halepensis*, resulting in a negative effect on the performance of native shrubs (Maestre et al., 2003).

Our results showed that reforestation of *P. halepensis* resulted in internode elongation and increased leaf length and width with increasing plantation age, whereas leaf length-to-width ratio decreased in older reforested plots. As mentioned above, *P. halepensis* dominates all reforested land and all other species by forming canopy overhangs for native species and significantly reducing light under the canopy. Changes in light gradients within the canopy of different species and forest types produce changes in leaf traits (Ugarte et al., 2010). Leaf adaptation to direct sunlight or shade occurs during leaf development and includes special morphological and biochemical adaptations (Lichtenthaler et al., 2007). In shade-avoiding plants, a series of morphological adaptations help to outperform competitors, such as elongation of stem-like structures, elevation of leaves, reduction of branching, and acceleration of flowering (Ugarte et al., 2010). Our study showed that all four species (*Quercus ilex L., Pistacia lentiscus L., Phyllyrea augustifolia L., and Cistus creticus L.*) recorded an elongation of internodes that increased with age and shading, and this was more pronounced for *Phillyrea augustifolia*. This internode elongation may increase plant height and improve light interception by the plant. Likewise, the effect of shade treatment on soybean plants showed a signifcant increase in stem length and petiole length (Wu et al., 2017). In shaded conditions, plants optimize photosynthesis and adapt to reduced light quality and quantity (Gommers et al., 2013). Therefore, they maximize light capture by increasing stem length and positioning leaves out of shade through a network of photoreceptor signaling (Franklin, 2008). This results in carbon being allocated to stem elongation at the expense of root and leaf development (Gommers et al., 2013). Plants have *a variety of* photosensory receptors to detect the presence of competitors and thereby adjust their growth and developmental strategies accordingly (Fiorucci & Fankhauser, 2017).

Our results showed an increase in the length and width of leaves in reforested plots, so an increase in the leaf area, for all four species. Indeed, plants can also adopt shade-tolerant strategies by increasing leaf area to maximize photosynthesis. Leaves tend to increase their leaf area in the shade to intercept more light (Clendon & Millen, 1982). Whereas, in the shade treatment, the total leaf area and leaf size of soybean plants were significantly smaller than those of the full sun control plants. Furthermore, the number and size of leaf cells were significantly reduced in the shade (Wu et al., 2017). Our results also indicated that increasing leaf width was more important than increasing leaf length, with leaves in reforested plots becoming larger rather than longer compared to control samples in native forest, especially for *Quercus ilex.* According to Eckardt et al., 1978 *Quercus ilex* is considered a shade-tolerant tree species and becomes the dominant tree species in the later stages of succession, with photosynthesis occurring year-round. It is a shade-tolerant species regenerating under the canopy cover (de Rigo & Caudullo, 2016). On the other hand, *P. halepensis* could act on other species by producing secondary metabolites (terpenes and phenols) that are defensive and therefore participate in the competitive mechanism (Bonin et al., 2007). Plants introduce allelochemicals into the environment through foliar leaching, root exudation, residue decomposition, volatilization, and debris incorporation into soil (Inderjit & Keating, 1999). These allelochemicals can alter physiological processes (e.g., chlorophyll accumulation, photosynthesis, nutrient uptake, cell division, or elongation (Inderjit & Duke, 2003). Indeed, the introduction of *P. halepensis* with afforestation has adversely affected existing late-successional shrubs and suggests that this introduction does not stimulate successional processes in Mediterranean semiarid areas (Bellot et al., 2004). To reduce the ecological risks of afforestation projects, the Convention on Biological Diversity guidelines recommend planting native woody tree species, with the aim of increasing the similarity between planted forests and native forests. However, reforestation with exotic tree species can be beneficial in ecosystem restoration, and sustainable management of the sustainable forest management. Exotic species can sometimes provide food and habitat for the native fauna and help to establish forest cover and soil stabilization more quickly. However, care should be taken to select species with low potential for invasion (Reisman-Berman et al., 2019).

Conclusion

The objective of this work is to study the impact of *Pinus halepensis* reforestation on the above-ground biomass and morphological characteristics of native species (internode elongation and leaf size) in the Beni Sohane Forest. The fast growth of *P. halepensis* trees formed a canopy above all indigenous species resulting in a significant decrease in the weight of dry matter above-ground biomass. This has led to the abandonment of pastors of important pastures previously exploited by cattle. The P. halepensis reforestation resulted in changes in internode elongation and leaf size of four native species (*Quercus ilex L., Pistacia lentiscus L., Phyllyrea augustifolia L., and Cistus creticus L.*). For the two oldest plantations (± 25-year-old and ± 45-year-old), the internode length, leaf width, and length of the 4 species have been significantly increased. However, the leaf length-to-width ratio decreased significantly, with leaves in reforested plots being larger rather than longer compared to control samples in native forest, especially for *Quercus ilex*.

Regular thinning of the *P. halepensis* trees would be necessary to restore the balance of these ecosystems. Thus, species selection for reforestation should consider maximizing rather than destroying ecological and socioeconomic services.

Conflict of Interest: The authors reported no conflict of interest.

Data Availability: All data are included in the content of the paper.

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