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## Unravelling the influence of light, litter and understorey vegetation on *Pinus pinea* natural regeneration

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*Pinus pinea* (L.) is one of the most valuable species used in the Tunisian reforestation programme, with about 21 000 ha of plantations. In the coming decades the oldest of these stands will begin their regeneration stage. However, little is known about the factors that control the natural regeneration of this species. It is reputed to be a strict shade-intolerant species and so needs light to regenerate satisfactorily. Regeneration can also be influenced by understorey vegetation and litter, both correlated with light availability. The aim of this study was to quantify the respective importance of these three factors in *P. pinea* regeneration. Live seedlings were counted in 90 plots (500 m<sup>2</sup> each) in three forests of *P. pinea* located in the coastal dunes in north Tunisia, and related to light availability, biomass of understorey vegetation and litter. In addition, the influence of litter was experimentally manipulated by creating 1 m<sup>2</sup> regenerating areas free of litter, with a light litter layer or the natural thickness. The density of 1-year-old pine seedlings was controlled mainly by litter biomass, whereas light availability increased the growth of older seedlings. Understorey vegetation did not appear to play a significant role in *P. pinea* regeneration in these Mediterranean climatic conditions. Management for natural regeneration of *P. pinea* should include scarification to reduce litter thickness and heavy thinning to significantly increase light availability.

### Introduction

Understanding the factors driving the distribution of species and their abundance is an important research area in ecology, since it refers to species coexistence and the maintenance of species diversity (Chesson, 2000). Ecological mechanisms proposed to explain community assemblage and the maintenance of biodiversity are (1) the niche assemblage model that views local communities as deterministic, in which local environment conditions and biotic interactions influence the assemblage, diversity and composition of communities (Hutchinson, 1957; Chesson, 2000; Hubbell, 2001; Chase and Leibold, 2003) and (2) the dispersal-assemblage model that views local communities as stochastic assemblages, in which the size of species pool, colonization hazards and immigration history primarily influence community assemblage (MacArthur and Wilson, 1967; Bell, 2001; Hubbell, 2001). Although theory suggests an important role of dispersal assemblage in high-diversified communities, membership in local communities may also depend on the ability of species to tolerate niche-based 'ecological filters' imposed by local abiotic or biotic conditions (e.g. Keddy, 1992; Diaz *et al.*, 1998; Myers and Harms, 2009).

The seedling recruitment stage is a key component of the regeneration niche (Grubb, 1977) that determines the initial success or failure of a species to establish (Gibson and Good,

1987). This process determines local community composition at a given site, and can be limited by a number of factors (Clark *et al.*, 1999). Recruitment may fail due to insufficient seed dispersal and fecundity (Barot, 2004). Niche limitation reflects local abiotic and biotic filters which restrict establishment of species that have successfully dispersed into a site (Weiher and Keddy, 1999; Hobbs and Norton, 2004; Tilman, 2004). Dispersal limitation may be the dominant constraint to diversity and community structure during the early stages of succession (Standish *et al.*, 2007; Mathias *et al.*, 2009), while niche limitation and biotic interactions may increase in importance as succession proceeds (Levine *et al.*, 2004). In this context, Clark *et al.* (1999) suggested that studying these filters (dispersal assemblage, niche assemblage) and their interactions is necessary to assess their full impact on community composition.

When studying the effects of biotic factors, it is important to consider both competitive and facilitative interactions (Forrester *et al.*, 2011). Several theoretical models predict that the relative importance of facilitation and competition should vary inversely. According to the stress-gradient hypothesis (SGH), facilitative interactions are more likely to dominate competitive interactions towards more harsh ends of environmental gradients where one species or cohort can act as a nurse for another (Bertness and Callaway, 1994; Callaway and Walker, 1997; Brooker and Callaghan, 1998). In addition to these spatial effects, shifts

between facilitation and competition have also been observed through time. These temporal dynamics result from changes in the relative sizes of co-occurring cohorts or species, their influence on growing conditions and microclimate fluctuations (Reisman-Berman, 2007; Armas and Pugnaire, 2009).

The stone pine (*Pinus pinea* L.) is one of the most valuable species in Tunisian reforestation programmes for its ecological uses (erosion control) and aesthetic and economic value (wood production, cone yields for pine nuts, resins, etc.). Tunisia presently has about 21 000 ha of stone pine plantations, and in the coming decades the oldest of these stands will begin their regeneration stage. However, little is known about the factors that control the natural regeneration of this species, and difficulty in regenerating this species naturally has been reported in Greece (Ganatsas *et al.*, 2008) and Spain (Calama and Montero, 2007; Barbeito *et al.*, 2008). *Pinus pinea* is considered to be a species easily propagated by seeds (Ganatsas and Tsakalidimi, 2007); however, successive filters may prevent its establishment and early growth—including variability in seed production, litter, light and understorey vegetation. Several hypotheses concerning these difficulties have been proposed, including (1) high variability in seed germination of this species depends on environmental conditions and population variability (Skordilis and Thanos, 1997; Escudero *et al.*, 2002), (2) the narrow optimal conditions for seed germination and seedling development (Magini, 1955) and (3) insufficient supply or low quality of the seeds produced by mother trees (Ganatsas *et al.*, 2008). Cone-yield variations are mostly due to climatic factors, particularly water stress (e.g. Mutke *et al.*, 2005). Seed morphology and size vary widely according to stand condition (site index, density, age and crown size; Calama and Montero, 2007), but potential seed germination rate is almost always found to be high (78–98%; Masetti and Mencuccini, 1991; Ganatsas *et al.*, 2008). Less is known about how local conditions, both abiotic (temperature, humidity and light) and biotic (needle litter, understorey vegetation and herbivory), affect seed germination and seedling establishment.

Among the different factors controlling the stone pine regeneration, the present study focussed on the seedling early establishment stage, as the available data on the main factors governing it are sparse. We hypothesized that stone pine seedling establishment was mainly controlled in order of importance by (1) light availability, (2) amount of needle litter and (3) competition with understorey vegetation. To evaluate these interactions, we examined (1) correlation among site variables (light, litter biomass and understorey vegetation) and (2) their effects on seedling early establishment.

## Materials and methods

### Study sites

The study was conducted in the north of Tunisia, in three sites afforested with *Pinus pinea* L. 20–50 years ago, and designated Mekna III (36°57'N, 8°45'E), Ouchtata II (36°59'N, 9°03'E) and Bechateur (37°16'N, 9°52'E), located in coastal dunes of Tabarka, Nefza and Bizerte, respectively. The whole area is characterized by sandy dune formations overlying limestone (Ouchtata II and Bechateur) and sandstone bedrock (Mekna III). The topography is relatively flat in Mekna III with a mean elevation of 12 m, whereas slopes at Ouchtata II range from 10 to 45% and from 10 to 85% in Bechateur, with a mean elevation of 80–112 m, respectively. Typical soil types found in the area include small quantities of

limestone in Ouchtata II, limestone and sometimes clay in Bechateur and sandstone in Mekna III. Soil fertility is poor with low total N content. Soils show very low organic matter levels (<0.5%). The C/N ratio is very high in Mekna III and Ouchtata II (25) and lower in Bechateur (18). The pH is basic in the three sites, 7.77, 7.82 and 8.6 in Bechateur, Ouchtata and Mekna III, respectively. The climate is Mediterranean with long summer dry periods 4–6 months long. Rainfall is concentrated during the winter (43.7%) and autumn (33.4%) in Mekna III. However, in Ouchtata II it is particularly rainy from September to April with a maximum in December (178.1 mm). In Bechateur, the rainfall period is between October and April, peaking in December (107 mm) and January (116.4 mm). The maximum mean temperature of the hottest month is 32.4, 36.1, and 32.7°C in Mekna III, Ouchtata II and Bechateur, respectively. The minimum mean of the coldest month is 7.9°C in Mekna III, 7.2°C in Ouchtata II and 7.3°C in Bechateur.

*Pinus pinea* was the dominant overstorey species in all the three forests. Deficiency of natural regeneration of *P. pinea* has been observed for many decades in these forests. Several explanations are given by foresters besides abiotic or biotic factors: first, the absence of an appropriate silviculture to promote cone and seed production; secondly, illegal cone collection by inhabitants and lastly, damage may be caused by overgrazing. Unfortunately, no management records of harvesting operations or thinnings are available for these plantations. However, some stumps were observed, indicating that sites had been partially harvested.

The main woody species present in the understorey were *Juniperus oxycedrus* ssp. *macrocarpa* Ball, *J. phoenicea* L., *Quercus coccifera* L., *Pistacia lentiscus* L., *Olea europea* L., *Arbutus unedo* L. and *Daphne gnidium*. There was also a herbaceous layer consisting of *Bellis annua* L., *Briza maxima* L., *Geranium robertianum* L., *Silene colora* ssp. *colorata*, *Anagalis arvensis* L., *Lagurus ovatus* L. and *Brachypodium pterococa*. The forest floor of the three forests is mainly needles and partially decomposed needle litter with a whole thickness between 2 and 6 cm in Ouchtata II and Bechateur and 2 and 8 cm in Mekna III.

### Experimental design and measurements

Stone pine regeneration was sampled in 90 rectangular plots (25 m × 20 m), distributed in the three forests (~30 plots in each forest), in order to sample the whole forest variability relative to stand characteristics (mainly age and density, Table 1). The forest of Bechateur presented the youngest stands, with a mean age of 28 years (range 19–38), the lowest height (range 5–17 m) and the highest mean density (862 trees ha<sup>-1</sup>).

For each plot, the total number of pines with a diameter at breast height (DBH) >5 cm (*N*) was counted and their DBH was measured. Tree age was determined by counting whorls. Total stem height and crown diameter (CD) were measured on a subsample of five stone pines, one in the centre and the others close to the four corners of the 500 m<sup>2</sup> plot to sample the whole variability of the plot, which always remained low (even-aged stands). CD was calculated as the average of

**Table 1** Stand characteristics of the three coastal *Pinus pinea* forests located in north Tunisia (mean value ± standard deviation)

Site	Density (number of trees ha <sup>-1</sup> )	DBH (cm)	Height (m)	Age (years)	
				Min	Max
Mekna	462 ± 352	29.6 ± 10.3	12.8 ± 3.7	20	50
Ouchtata	576 ± 401	26.4 ± 8.0	12.0 ± 2.8	18	36
Bechateur	862 ± 301	20.2 ± 6.7	9.0 ± 2.4	19	38

two values measured in two perpendicular directions, N–S and E–W, by projecting the edges of the crown to the ground and measuring length along one axis from edge to edge. Overstorey canopy cover ( $C$ , %) was then calculated by:

$$C = \text{PCA} \times N \times \frac{100}{\text{plot surface}} \quad (1)$$

where PCA is the mean projected crown area derived from  $\pi \times (\text{CD}/2)^2$ .

All live stone pine seedlings (height <1.5 m) were counted in each plot. In order to ensure not to miss any seedlings, each plot was divided into 20 subplots (25 m<sup>2</sup>). Their age was estimated by counting whorls (one each year). Seedling mortality was not recorded.

The cover (in %) of dominant understorey species (shrubs with height ≤1m, small trees with height >1 m but not including young stone pine trees, or herbaceous species) was estimated by the visual projection of the whole foliage onto the soil in each plot. The understorey biomass was measured on samples (selected to account for the entire cover range) of each dominant species, cut and transported to the laboratory for dry weight determination after oven drying at 88°C. Total biomass per plot was then calculated from total per cent cover of these species. Needle litter was collected in eight square subplots (0.5 m × 0.5 m) distributed on the plot (both under and between trees) and brought to the laboratory for dry weight determination. Total litter biomass of the plot was then extrapolated from the mean of the eight samples.

Light availability in the understorey was estimated by hemispherical photographs. Five photographs per plot were taken every 5 m along a line crossing the plot to sample the variability of light due to the possible presence of small gaps. The equipment used was a Nikon F70 camera with a Sigma 8 mm 1:4DG EX fisheye lens and a 180° aperture. The colour image was converted into a black (vegetation) and white (sky) picture, using the PfiPhotem software to threshold the image (Adam *et al.*, 2006). The transmittance was then calculated using PfiLA (Adam *et al.*, 2008) and averaged for two periods: 15 September to 15 October and 15 March to 15 April, the periods of seed germination. The proportion of direct and diffuse light was 0.69:0.31 for Mekna III, 0.59:0.41 for Ouchtata II and 0.72:0.28 for Bechateur. Stand transmittance ( $T$ , %) was then calculated as the mean of the five photographs.

An additional experiment was conducted to specifically address the effect of litter. For each forest (Mekna III, Ouchtata II and Bechateur), five 1 m<sup>2</sup> subplots were established in October 2010 with a factorial design comprising three forest floor conditions: bare soil with total forest floor removal, thin forest floor layer with removal of some of the upper forest floor layers (ca. 3 cm) and natural forest floor thickness (ca. 6 cm). Three replicates were used for each condition. Thus, a total of 135 subplots were set up (3 replicates × 3 litter conditions × 5 plots in each forest × 3 forests). Stone pine seedling emergence on each subplot was surveyed in May 2011.

### Statistical analysis

Data analysis was performed using Statgraphics Centurion XV (StatPoint, Inc., Virginia, USA). As the response of the stone pine seedlings to different variables was age-dependent, analyses were separately performed (1) on 1-year-old seedlings to test for conditions controlling seedling emergence and (2) on seedlings >1 year to analyse factors controlling subsequent seedling survival and growth.

The different variables were first subjected to a Spearman's rank correlation analysis to determine the main links between them (data not shown). As the different variables were not totally independent, partial correlations were also calculated to measure the link between two variables, taking into the account the link with the other variables. From those data, the links were then more specifically analysed by general regressions. The dependent variables were log (natural

logarithm) – transformed when necessary to correct the non-randomness of residues. Regressions were used in particular to link density of 1-year-old seedlings to transmittance, litter biomass and stand age. However, such an analysis was impossible for seedlings >1 year, because there were a large number of plots with no seedlings. Therefore, a non-parametric Kruskal–Wallis comparison test based on ranks was used to identify the significant differences among transmittance and litter biomass data after the transformation of the independent variables into classes, the size of every class being set to have an equal number of individuals. The effect of the different transmittance classes on understorey biomass was also determined after a non-parametric Kruskal–Wallis comparison test followed by a Mann–Whitney test based on the median to identify significant differences among classes. For these different analyses, a site effect was tested, but had no significant influence on the results ( $P = 0.22$ ), so that subsequent analyses were performed by pooling the data from the three sites.

Finally, ANOVA was used to identify the influence of the three litter conditions (bare soil, moderate density of litter and thick litter) on seedling establishment in the experimental approach. Litter condition was used as fixed effect and site as a random effect. For variance homogenization, seedling density was square-root-transformed (but data in Table 4 are presented without transformation, for clarity).

## Results

### Light availability and stand features

The stand transmittance ( $T$ , %) ranged from  $6.2\% \pm 0.08$  to  $31\% \pm 0.01$  depending on the overstorey cover ( $C$ , %):

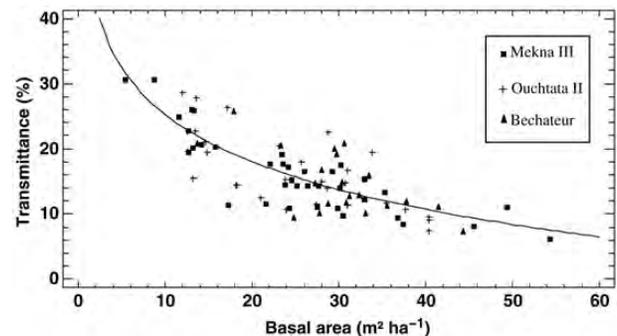
$$T = \frac{7.71 + 670}{C}, \quad R^2 = 0.63, \quad P < 0.0001, \quad (2)$$

and also decreased significantly with increasing stand basal area ( $G$ , m<sup>2</sup> ha<sup>-1</sup>) (Figure 1)

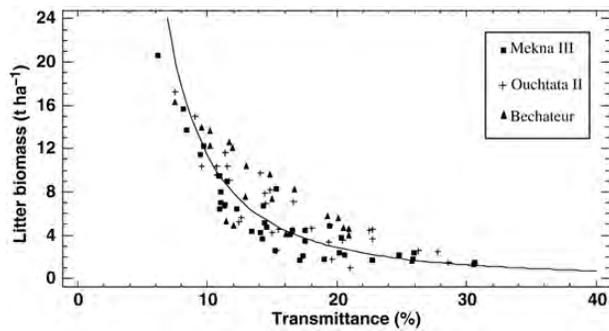
$$T = 49.22 - 10.43 \times \log(G), \quad R^2 = 0.63, \quad P < 0.0001. \quad (3)$$

Litter biomass (LB, kg ha<sup>-1</sup>) was positively correlated with the overstorey cover:

$$\text{LB} = (0.25 \times \sqrt{C})^2, \quad R^2 = 0.96, \quad P < 0.0001, \quad (4)$$



**Figure 1** Mean stand transmittance relative to stand basal area ( $G$ , m<sup>2</sup> ha<sup>-1</sup>) in three forests of *Pinus pinea* in the coastal dunes of north Tunisia [equation (3): transmittance =  $49.22 - 10.43 \times \log G$ ].



**Figure 2** Litter biomass relative to transmittance in three forests of *Pinus pinea* in the coastal dunes of north Tunisia [equation (5): litter biomass =  $(33.77/T)^2$ ].

**Table 2** Understorey biomass ( $\text{kg ha}^{-1}$ ) of understorey woody species, graminoids, forbs and litter relative to transmittance in three coastal *Pinus pinea* forests located in north Tunisia

Transmittance class	6.2–11.5	11.6–14.8	14.9–19.7	19.8–30.6
Woody species	223d <sup>1</sup>	1188c	3371b	17112a
Graminoids	4.95c	9.2c	34.6b	166.8a
Forbs	1.6c	5.2c	49.8b	151.5a
Litter	11440a	7426a	4390b	2909c

<sup>1</sup>For a given type of vegetation, different letters denote significant differences among transmittance classes according to the Mann-Whitney test at  $\alpha$ -level 0.05.

whereas a negative correlation was found with light transmittance (Figure 2 and Table 3):

$$\text{LB} = (33.77/T)^2, \quad R^2 = 0.97, \quad P < 0.0001. \quad (5)$$

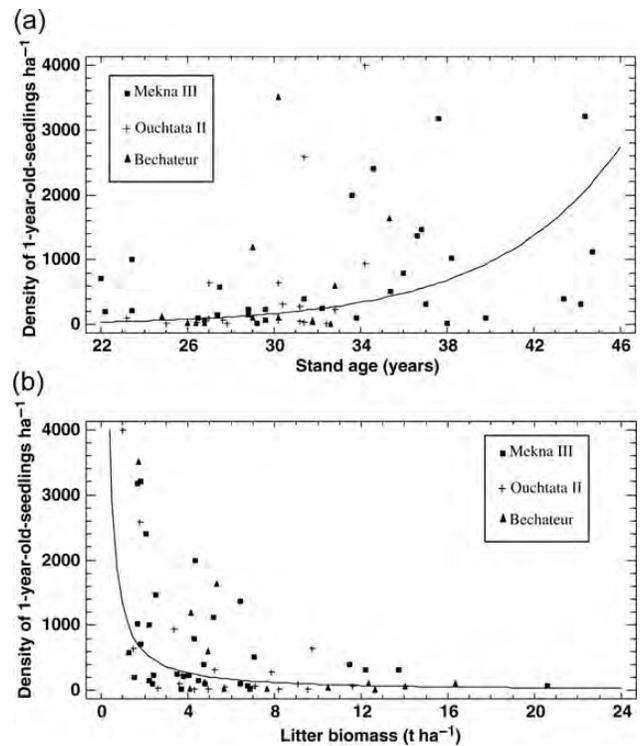
The biomass of understorey vegetation (woody species, graminoids and forbs) was found to be positively correlated with light transmittance ( $R^2 = 0.60$ ,  $P < 0.0001$ ;  $R^2 = 0.97$ ,  $P < 0.0001$ ;  $R^2 = 0.97$ ,  $P < 0.0001$ , respectively, Table 2). The lower transmittance class (6.2–11.5%) showed a lower understorey biomass, with 223  $\text{kg ha}^{-1}$  for woody species, 4.9 for graminoids and 1.6 for forbs. The understorey biomass was significantly greater under the upper transmittance class (19.8–30.6%) (17112  $\text{kg ha}^{-1}$  for woody vegetation, 167 for graminoids and 152  $\text{kg}$  for forbs).

### Stone pine seedling establishment

The older the stand, the higher the density of 1-year-old seedlings ( $D_1$ , number  $\text{ha}^{-1}$ ;  $R^2 = 0.93$ ,  $P = 0.0004$ ) (Figure 3a),

$$D_1 = \exp(0.17 \times \text{stand age}), \quad R^2 = 0.93, \quad P = 0.0004. \quad (6)$$

The use of partial correlations to factor in the links between transmittance and other variables showed that  $D_1$  was also firstly driven by litter biomass ( $r = -0.28$ ,  $P = 0.0009$ , Table 3a and Figure 3b) and to a much lesser extent by transmittance



**Figure 3** Density of 1-year-old stone pine seedlings (number  $\text{ha}^{-1}$ ) according to stand age (a) and litter biomass (b) in three forests of *Pinus pinea* in the coastal dunes of north Tunisia [equation (6): density of 1-year-old seedlings =  $\exp(0.17 \times \text{stand age})$ ; equation (7): density of 1-year-old seedlings =  $\exp(7.15 - 1.12 \times \log(\text{litter biomass}))$ ].

( $r = 0.23$ ,  $P = 0.035$ ) (Figure 3b):

$$D_1 = \exp(7.15 - 1.12 \times \log(\text{LB})), \quad R^2 = 0.26, \quad P < 0.0001. \quad (7)$$

The understorey vegetation seemed to play no role in  $D_1$  ( $P > 0.05$ , Table 3a).

The density of stone pine seedlings  $>1$  year ( $D_{>1}$ , number  $\text{ha}^{-1}$ ) was positively influenced by transmittance ( $r = 0.34$ ,  $P = 0.002$ , Table 3b) with a possible threshold effect (almost no seedlings were found below 20% transmittance, data not shown) and negatively by the woody understorey vegetation ( $r = -0.29$ ,  $P = 0.008$ ). Litter biomass had no influence on  $D_{>1}$  ( $P > 0.05$ ).

### Specific effect of litter on *P. pinea* seedling emergence

The manipulation of litter in 1  $\text{m}^2$  area showed that the lowest rate of stone pine seedling emergence was recorded for the thickest litter condition in all the three forests (Table 4). A bare soil or a thin litter layer bore significantly more seedlings, the two conditions being equivalent in Bechateur. The thin litter layer bore more seedlings than bare soil in Mekna III and Ouchtata II (Table 4), but the opposite trend was found at Bechateur.

**Table 3** Partial correlations (level of significance) between density of 1-year-old stone pine seedlings (a) and >1 year (b) and main variables of stands in three coastal *Pinus pinea* forests located in north Tunisia

	Transmittance	Litter biomass	Woody species biomass	Graminoid biomass	Forb biomass
(a)					
1-year-old seedling density	<b>0.23 (0.035)</b>	<b>-0.28 (0.009)</b>	-0.19 (0.08)	-0.07 (0.54)	0.0015 (0.99)
Transmittance		-0.18 (0.09)	0.12 (0.28)	<b>0.27 (0.012)</b>	<b>0.30 (0.005)</b>
Litter biomass			0.04 (0.69)	0.06 (0.57)	-0.13 (0.24)
Woody species biomass				0.08 (0.45)	<b>0.38 (0.0004)</b>
Graminoid biomass					<b>0.56 (0.00001)</b>
(b)					
>1 year seedling density	<b>0.34 (0.002)</b>	0.004 (0.97)	<b>-0.29 (0.008)</b>	0.16 (0.15)	0.09 (0.41)
Transmittance		<b>-0.25 (0.02)</b>	0.17 (0.12)	0.19 (0.08)	<b>0.26 (0.02)</b>
Litter biomass			0.10 (0.37)	0.08 (0.45)	-0.14 (0.21)
Woody species biomass				0.14 (0.21)	<b>0.39 (0.0002)</b>
Graminoid biomass					<b>0.54 (0.00001)</b>

Significant partial correlations are in bold.

**Table 4** Number of stone pine seedlings recorded in May 2011 on 1 m<sup>2</sup> areas set up in October 2010 free of litter, with a thin litter layer or a thick litter layer in three coastal *Pinus pinea* forests located in north Tunisia

Litter condition	Mekna	Ouchtata	Bechateur	P
Bare soil	3.6 (b) (A) <sup>#</sup>	3.9 (b) (AB)	2.4 (a) (B)	0.09
Thin litter	5.5 (a) (A)	5.8 (a) (A)	3.0 (a) (B)	<0.0001
Thick litter	0.3 (c) (A)	0.4 (c) (A)	0.3 (b) (A)	0.71
P	<0.0001	<0.0001	<0.0001	

<sup>#</sup>Different lower-case letters indicate significant differences between conditions for a same site. Different upper-case letters denote significant differences between sites for the same condition.

## Discussion

### Assessing stand light availability

The stone pine (*Pinus pinea* L.) is reputed to be a very light-requiring species, and so light is a fundamental factor for many processes linked to its development, but characterizing this resource is often difficult. Direct measurements with sensors or hemispherical photographs as in this study are possible, but they are costly or time-consuming or both. Spatial variability in understorey light is largely determined by several characteristics of overstorey plants (Scott *et al.*, 2000), and so crown projection maps and gap sizes were used in some previous studies to estimate relative light conditions on the forest floor (Yamamoto, 1993, 1995). Other authors published work underlining closer relationships between light transmittance and other common indirect measures such as canopy cover (Johansson, 1996) or basal area (Sonohat *et al.*, 2004; Balandier *et al.*, 2006). In our study, two predictive measures, overstorey cover and stand basal area, showed significant correlations with light transmittance, and both had  $R^2$  values of 0.63. These values are lower than those reported in other studies due to a narrow range of variation (transmittance from 6 to 31%), but the

relationships can be used to assess light in the understorey of *P. pinea* stands.

### Stone pine seedling emergence and survival

Our first hypothesis was that light would be the main factor controlling stone pine seedling emergence and growth. Actual results showed that light had only a limited influence on the abundance of *P. pinea* seedlings  $\leq 1$  year old, but significantly influenced the recorded number of seedlings >1 year. In fact the results suggest that the stone pine seed germination and seedling emergence *stricto sensu* do not need a high light level, whereas subsequent seedling survival and growth is mainly determined by light. This is in line with previous studies by Ganatsas and Tsakalimi (2007), who found that percentage germination of *P. pinea* seed was high and did not depend on light conditions, and with Fady *et al.* (2004), who found that the species is light-demanding after the germination stage. In a Mediterranean climate, moderate cover could protect seedlings of stone pine from desiccation and promote germination (Adili *et al.*, unpublished data).

In this investigation, *P. pinea* seedlings >1 year were absent in very small gaps (transmittance <20%). Consequently, after germination and when seedlings are established, light becomes the main factor driving seedling survival. Gaudio *et al.* (2011) showed with *P. sylvestris* that the light requirement increased with the size of individuals; when very small, they were able to survive deep shade, but their light requirements rapidly increased as they grew.

The results obtained here indicate that litter had a negative effect on stone pine seedling emergence and early establishment (1-year-old seedling) and this negative effect increases with increasing litter thickness (Facelli and Pickett, 1991). The forest floor/litter appears to be a selective barrier to emergence when seeds are dispersed onto top of the litter layer in two ways: (1) mechanically by preventing seed radicle from reaching the mineral soil surface (Facelli and Pickett, 1991; Caccia and Ballaré, 1998; Wilby and Brown, 2001) and (2) physiologically

through seed desiccation limiting the seed imbibition process (Adili *et al.*, unpublished data). In addition, allelopathic inhibition may explain failure in conifer regeneration (Mallik, 2003). An allelopathic effect of litter was observed with old *P. halepensis* forests (Fernandez *et al.*, 2008). However, our investigations with litter extracts show no evidence of allelopathic mechanisms controlling the emergence of *P. pinea* (Adili, unpublished data).

The specific and relative roles of litter and light were difficult to disentangle *a priori* because, litter quantity increases with increasing canopy cover and decreases with increasing light availability. Consequently, litter biomass was linked to light transmittance. However, the partial correlation analyses clearly showed that litter was the primary factor that controlled seedling emergence, whereas light was the main factor controlling subsequent seedling growth. Thus, better seedling emergence seems to be linked with decreased litter accumulation or rapid decomposition, and subsequent seedling growth is likely to be improved by increased light availability. Jiao-jun *et al.* (2003) report the same results on *P. thunbergii*.

Plant litter has differential effects on plant performance at different amount and at different life stages (e.g. Koorem *et al.*, 2011). Germination and early establishment are two key stages in plant community assembly (Grubb, 1977) that are particularly sensitive to the presence of litter (Facelli and Pickett, 1991). Generally, the effect of litter on seedling early establishment is negative, and this negative effect increases with increasing amount (Facelli and Pickett, 1991; Xiong and Nilsson, 1999). In our study, negative effect of litter seems to be more important on the density of 1-year-old seedlings ( $P=0.009$ ) than on the density of seedlings >1-year ( $P>0.05$ ), suggesting that litter have mostly negative effect on emergence contrasting with a neutral-to-positive effect on subsequent seedling growth.

### Understorey vegetation influence on seedling density

As in other studies (Lieffers and Stadt, 1994; Riegel *et al.*, 1995; Griffith, 1996; Ricard and Messier, 1996), understorey vegetation biomass (woody species, graminoids and forbs) was positively affected by increasing light availability. Many experimental studies in Mediterranean environments (Gómez *et al.*, 2001a; Maestre *et al.*, 2001; Castro *et al.*, 2002) reported a facilitative effect of shrubs and forbs on the early establishment of seedlings of different woody species, either by buffering microclimatic conditions or by protecting seedlings from herbivore damage (Rousset and Lepart, 1999; Callaway, 1995; García *et al.*, 2000; Gómez *et al.*, 2001b). However, in this study, even when well developed, the understorey vegetation (graminoids, woody species and forbs) had no noticeable effect on the emergence of *P. pinea* seedlings in any experimental site. Montgomery *et al.* (2010) showed a dependence of the interactions between understorey vegetation and tree seedlings with the structure of the overstorey. In our study, any facilitative effect of understorey vegetation on seedling early establishment may be masked by the dense overstorey cover, which prevented a strong development of the understorey vegetation and which presumably buffers microclimatic conditions.

An understorey woody vegetation did affect the survival of pine seedlings >1 year, presumably by competing for soil moisture and nutrients (e.g. Mesón and Montoya, 1993; García-Salmerón, 1995; Serrada, 1995). The nature and extent of

understorey woody vegetation-seedling interactions (facilitation vs competition) on these sites is likely to change with size and seedling development and would depend on ecological context (e.g. overstorey cover) (e.g. Montgomery *et al.*, 2010).

### Effect of stand age on pine seedling density

Increasing stand age was found to favour 1-year-old stone pine seedling density, probably as cone and seed production increase with tree age (Cappelli, 1958; Magini, 1966). Cone production of *P. pinea* begins late, at age 15–20 years, compared with other Mediterranean pine (10–15 years or earlier for *P. halepensis*, *P. brutia*, *P. pinaster*; Thanos and Daskalaku, 2000; Tapias *et al.*, 2001; Zagas *et al.*, 2004; Ganatsas *et al.*, 2008). Therefore, our forest stands, with a mean age of 30 years, may only be commencing their reproductive years.

## Conclusion and implications for stone pine regeneration management

Litter thickness was the main factor controlling stone pine seedling emergence and early establishment, whereas light was necessary for subsequent seedling growth. Therefore, a management plan of natural regeneration of *P. pinea* should first include scarification to reduce litter thickness (Harrington and Edwards, 1999; Montero *et al.*, 1999; Nadelhoffer *et al.*, 2000) and then heavy thinning to significantly increase light availability while retaining good-quality parent trees for seed dispersal. This could be conducted within the context of a shelterwood silvicultural system. The role of understorey vegetation and especially of woody species is more complex. These understorey plants may provide a microclimate moderation in the stage of seedling early establishment, but may be detrimental to subsequent seedling growth. As an initial approach, a litter thickness of <3 cm and a light availability of at least 20–30% of above-canopy irradiance can be recommended.

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