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RESEARCH ARTICLE

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Restoration of European yew (*Taxus baccata* L.) in Mediterranean mountains: importance of seedling nursery fertilization and post-planting light levels

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Abstract

Aim of the study: We studied the influence of nursery fertilization and post-planting light environment on the growth and survival of out-planted two-year-old yew (*Taxus baccata*) seedlings.

Area of study: Post-planting performance was assessed at two sites in the Valsain Forest (Central Mountain Range, Segovia, Spain).

Materials and Methods: Seedlings were grown using the same seed-lot, container type and fertirrigation schedule. A soluble fertilizer with two contrasting doses resulting in 239 and 376 mg N per seedling was applied during the whole culture period. Seedlings grown under the highest level of fertilization had greater root collar diameter, height, shoot to root ratio, root and shoot mass, and root growth potential before planting. Post-planting performance was assessed at two sites in the Valsain Forest (Central Mountain Range, Segovia, Spain). In each site, seedlings from both fertilization treatments were planted in three plots with contrasting light environment (full sunlight, and under *Pinus sylvestris* stands with moderate and deep shade conditions). Survival, diameter and height growth were monitored for six years.

Main results: Nursery fertilization did not affect survival, but high fertilization enhanced post-planting growth. Survival was highest under deep shade conditions but growth in this microsite was lower than in higher light sites, revealing a trade-off for survival and growth across light levels.

Research highlights: The lower fertilization rate used in this study was suitable to produce seedlings with acceptable quality. Planting under shaded conditions (light availability<30%) is recommended to maximize the initial success of yew plantations in Mediterranean mountains.

Keywords: *Taxus baccata*; plant quality; field growth; survival; reforestation.

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Introduction

European yew (*Taxus baccata* L.) is an endangered forest species, currently forming small isolated populations in many parts of Europe (Thomas & Polwart, 2003), which are listed as a "priority habitat" by the European Union to ensure their persistence. In the Iberian Peninsula, although yew has a wide distribution (Oria de Rueda, 1997), it is not a dominant tree, and it appears in the understory of forests dominated by other main tree species. Yew population decline has been attributed to felling, controlled burnings to prevent livestock poisoning, seed and seedling herbivory (by deer mainly), and regeneration limitations associated to dioecy and isolation of individuals (Iszkulo, 2010).

In addition, restoration and conservation of populations using planted seedlings has been limited due to poor seed germination due to persistent dormancy. Yew is usually propagated from cuttings, which reduces genetic diversity of restored populations. Moreover, studies about nursery production are rare, and generally focused on ornamental production (Khatamian &

Lumis, 1982; Lis-Krzyścin, 2010), which cultivation methods and plant quality requirements are different from those for restoration practices (Chirino *et al*., 2009).

In the Mediterranean basin, nursery cultivation has been demonstrated to be an effective tool for enhancing plantation success in restoration and conservation projects. Nitrogen fertilization in the nursery usually increases survival and growth of out-planted seedlings (Oliet *et al*., 2009; Villar-Salvador *et al*., 2005; Villar-Salvador *et al*., 2012). However, as far as we know, there is no knowledge about the nutritional requirements of yew and how nursery N affects its post-planting performance.

In Spain, forest container and marketing of *T. baccata* is regulated by law (RD 289/2003). However, in contrast to other more widespread planted trees, yew seedling quality prescriptions for forest plantations have not been defined. For this reason, studies are needed for increasing knowledge on nursery production and plant quality references for yew.

Yew is considered to be a shade-tolerant species (Brzeziecki & Kienast, 1994; Thomas, 2010), but the effects of light availability on survival and growth of young seedlings is not consistent across studies, with both positive and negative effects of light on seedling out-planting performance (Thomas & Polwart, 2003; Perrin & Mitchell, 2013; Iszkuło, 2010). Moreover, shading effects on post-planting performance may be different in southern regions of the distribution, where high light levels can interact with water stress, decreasing plantation success. (Linares, 2012).

Specifically, we assessed the influence of two levels of N fertilization in the nursery on several key morphological and plant performance quality attributes and on the post-planting response within three planting microsites differing in light conditions (in open sites, and under *Pinus sylvestris* stands with moderate and deep shade conditions). We hypothesized that high N nursery fertilization will result in high seedling quality and post-planting performance. Additionally, high light levels in the plantation environment will impair plant performance, and seedling quality would be more relevant under this environmental condition than under moderate or deep shade conditions. The aim of this work is contribute to the knowledge of pre-planting nursery fertilization and light environment in reforestation of yew seedlings.

Material and Methods

Seeds from a general stored collection sited in CNRGF "El Serranillo" were doubled- stratified according to the method proposed by Suszka (1985). Seeds were collected in Sierra de Guadarrama-Ayllón provenance (Central Spain). After germination, they were sown in forest containers (Plasnor, Legazpi, Spain), filled with fertilized peat moss (Kekkila 4460 B6 Finnpeat, Kekkila Ltd., Finland) during late December 2004. Each container had 45 cavities of 300 ml (density 283 seedlings m^{-2}). Plants were cultivated in a greenhouse until May 2005, and thereafter outdoors under full sunlight. Treatments were arranged in two blocks per treatment. In each block a fertilization treatment consisted of five containers (a total of 225 seedlings). Plants were watered three times a week.

In June, seedlings were randomly assigned to two N fertilization treatments, which were applied for two consecutive growing seasons: *F1* treatment, with 70 and 109 mg N per plant in the first and second season, respectively; and *F2* treatment, with 140 and 176 mg N per plant in the first and second season, respectively. The fertilization solution was made up with a 20-7-19 (N-P-K) commercial water-soluble fertilizer (Conifer Grower, Peters Professional®, Scotts). Nutrients were supplied by fertirigation and the fertilizer solution was applied weekly through 20 weeks in the first year, from early June to October 2005, and through 25 weeks in the second year, from June to December 2006.

At the end of the cultivation period in early 2007, 40 plants per fertilization treatment (20 plants per block) were randomly sampled for morphology (height, root collar diameter and mass) and tissue nutrient concentration (N, P and K).

On April 2, 2007, a root growth capacity (RGC) test was carried out. Twenty seedlings per treatment were transplanted into 3-l pots (Forest Pot, Nuevos Sistemas de Cultivo S.L., Girona, Spain) (one plant per pot) containing perlite. Seedlings were randomly arranged in a glasshouse for 23 days. Plants were then extracted, and the perlite was washed away and all new roots longer than 1 cm protruding from the root plug were cut and their mass measured after drying at 50º C for 48 h.

Two experimental plots were established in two sites under three light environments. The two sites were in the Monte de Valsaín forest (Segovia- Central Spain), which is the same provenance region of seed collection. In this forest, yew has natural populations. The first site (Site 1: Peña de las Tres Varas 1380 m a.s.l, 40º 52' 15.08" N and 3º 59' 30.82" W) has a gentle norther-faced slope. The second site was located at the top of a hill on a flat site (Site 2: Alto de la Fuente de los Pájaros, at 1529 m a.s.l, 40º 51' 33.48" N and 4º 3' 10.21" W). Both sites are within a *Pinus sylvestris* stand and distance between both sites is 5.5 km. Climate is Mediterranean continental with a mean annual air temperature of 9,7 and 9.0 ºC for site 1 and 2, respectively. Mean rainfall was 765 mm for site 1 and 890 mm for site 2 in the period 2007-2013. The duration of the vegetative period was 4 months in both sites. Soil is mainly sandy and pH=5.5 in site 1 and sandy loam, pH=5.8 with higher calcium concentration in site 2. In each sites, we selected three microsites, located <30 m from each other and defined by light availability due to tree cover. PAR photon flux density (PPFD, μmoles*m^{-2}*s^{-1}) was measured with a quantum sensor (Hansatech FMS2) to characterize three levels of light environment in relation to the radiation without tree cover: full sunlight, open canopy and closed canopy (95%, 30% and 5% of maximum PPFD, respectively). One plot per site and light environment was planted. In each plot, three replicates per fertilization treatment were planted in rows (10 seedlings per replicate, planted every 1 m within a row), with a total of 60 seedlings per light environment and site. Distance between rows was 2 m, with a total of 6 rows per plot.

Planting site preparation consisted in 80 cm depth holes that were excavated with a mechanical auger. Plots were fenced during the study to prevent damage by cattle and deer, which are very abundant in the area. Survival was recorded during the first year (in spring, summer and autumn) and after two and six years. Growth was recorded after one and six years. Height, root collar diameter (RCD) and stem volume increment was used as a measure of plant growth. Stem volume was calculated using height and RCD assuming that the stem is a cone.

Effect of fertilization on morphology, N concentration, RGC and field growth were analyzed by ANOVA, with container volume and block as fixed factors. Survival was analyzed using a generalized linear model with a binomial distribution and a logit link function. Due to high number of missing data, we selected 1 and 2 light environments to test the influence of nursery fertilization and illumination in the growth after one year in field at the two sites. Only light environment 3 was used to evaluate the growth after six years in field. Similarly, we selected data from Site 1 to analyze the effect of the light environment and nursery fertilization in the growth of seedlings after one and six years in field. Statistical analysis was performed with R software.

Results

At the end of the nursery cultivation period, fertilization had significantly affected morphology and root growth capacity (Table 1). RCD and both shoot and root mass was higher in *F2* seedlings that in *F1* ones. N fertilization also increased RGC. Shoot and root N concentration in *F2* seedling almost doubled the N concentration of *F1* seedlings.

Most outplanting mortality occurred during the first summer after planting (Figure 1). Site and light environment but not fertilization treatment significantly affected survival (Table 2). Seedlings planted at the lower altitude site had higher survival (Site 1: 83%, Site 2: 18%; p<0,001). Tree cover had a positive influence on survival. Survival after 6 years was significantly higher in closed canopy plots (76%) than in open canopy (41%) or at full sunlight (34%) (P<0,001), where had no showed significantly differences.

Height growth in the first year in field in Site 1 (3,20±1,35 cm; Table 3) was significantly lower than in Site 2 (0,94±0,36 cm). Light environment, considering data from open canopy and closed canopy (Table 3), significantly affected RCD growth in the first year

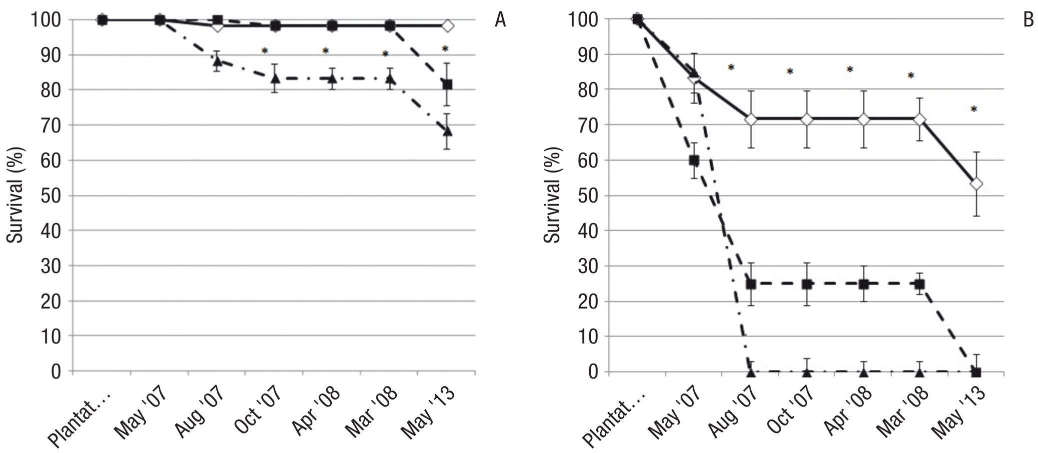

Figure 1. Field survival evolution for six years in each light environment. A: Site 1; B: Site 2. Light environment: 5% PPFD survival (◊); 30% PPFD survival (■); 95% PPFD survival (▲) .* Indicate significant differences (p < 0,05).

Table 1. Mean values ± one standard error and statistical results of morphology parameters, root growth capacity and N concentration of *Taxus baccata* seedlings at the end of nursery experiment. ANOVA one-way (Fertilization Factor) for all parameters.

	F1 Treatment	F2 Treatment	F	P value
Height (cm)	11,9±2,4	14,0±1,9	41,51	<0,0001
Root collar diameter (mm)	2,8±0,5	3,1±0,4	48,35	<0,0001
Root mass (g)	2,3±0,8	3,8±1,3	18,01	<0,0001
Shoot mass (g)	1,7±0,7	2,7±1	15,27	<0,0001
RGC (cm)	190,4±98,3	275,5±111,4	7,11	0,011
RGC (g)	0,1±0,05	0,19±0,1	14,27	<0,001
Shoot N concentration (%)	0,7±0,08	1,3±0,8	101,7	<0,0001
Root N concentration (%)	0,8±0,08	1,3±0,1	348,7	<0,0001

Table 2. Statistical results of the effect of nursery fertilization, shade environment and plantation site on plant survival after the first and sixth year.

Parameter	Survival first year (October 2007)			Survival sixth year (May 2013)		
	Log-Likehood	χ^2	*P-value*	Log-Likehood	χ^2	*P-value*
Fertilization	-237,4	0,428	0,513	-124,1	0,624	0,430
Light environment	-108,8	96,18	*<0,001*	-124,5	84,96	*<0,001*
Site	-156,9	161,13	*<0,001*	-166,9	165,19	*<0,001*

Table 3. ANOVA analyzing the height growth (cm) and RCD (mm) of the first year in field between type of nursery fertilization (F, levels F1 and F2), light environment (LE, closed and open canopy) and sites (S, 1 and 2).

Source of variation	*df*	RCD growth first year		Height growth first year	
		F	*P*	*F*	*P*
F	1	0,094	0,759	0,98	0,323
LE	1	4,187	*0,042*	0,75	0,388
S	1	0,148	0,701	88,25	*<0,001*
F x LE	1	1,34	0,249	0,15	0,955
F x S	1	0,014	0,905	0,23	0,629
LE x S	1	0,036	0,85	15,71	*<0,001*
F x LE x S	1	0,067	0,797	0,69	0,408

(RCD growth of open canopy=0,84±0,12 mm; RCD growth of closed canopy=0,68±0,05 mm). An interaction between light environment and site was found in height growth, where seedlings from upper site grown more in open canopy (4,02±1,02 cm) than in closed canopy (2,39±0,78 cm), and the opposite effect was found in lower site, with seedlings planted in open canopy increased less (0,59±0,12 cm) than in closed canopy (1,07±0,32 cm). No interaction between fertilization and site was found.

Height growth was influenced by light environment too (Table 4), where seedlings of open canopy (4,52±1,26 cm) grown more than in closed canopy (2,34±1,01 cm) or full sunlight (2,02±0,96 cm). After six years in field, the lower height (11,81±2,23 cm) and RCD growth (2,74±0,27 mm), was for seedlings planted in closed canopy, with no significant differences in height or diameter growth in the plants grown in full sunlight (height growth=34,03±3,81 cm; RCD growth=4,28±0,76 mm) or open canopy (height growth=33,15±2,67 cm; RCD growth=4,13±0,82 mm).

Height growth (*F1*: 19,35±2,03 cm vs *F2*: 24,27±1,44 cm) and RCD growth (*F1*: 3,06±0,09 mm vs *F2*: 3,59±0,30 mm) in field was largely enhanced by N fertilization after six years in field (Tabla 4). No interaction between fertilization and light environment was found.

Discussion

Afforestation with *Taxus baccata* is not an easy task because it is difficult to cultivate in the nursery; seeds have poor germination rates and seedlings grow slowly even if nutrient and water availability are optimal.

Table 4. ANOVA analyzing height growth (cm) and RCD (mm) in field between nursery fertilization (F, levels F1 and F2) and light environment (LE, closed canopy, open canopy and full sunlight). (For this analysis, only data from Site 1 –Peña de las Tres Varas- was used).

Source of variation	*df*	RCD growth 1st year		Height growth 1st year		RCD growth 6th year		Height growth 6th year	
		F	*P*	*F*	*P*	*F*	*P*	*F*	*P*
F	1	1,322	0,252	3,02	0,084	4,02	*0,047*	6,43	*0,012*
LE	2	1,722	0,182	19,33	*<0,001*	16,94	*<0,001*	77,95	*<0,001*
F x LE	2	1,324	0,269	0,43	0,648	2,11	0,125	0,05	0,952

Although a more detailed study on the effect of container volume on post-planting performance is needed, and specially to study if it´s possible to produce one year-old seedlings able to survive and grow adequately in the field under low stress planting conditions by growing yews in small volume containers (≤300 ml). Despite these nursery constraints, our results demonstrate that European yew can be propagated from seeds in forestry containers to produce seedlings of acceptable quality, which are able to perform quite well in Mediterranean mountain environments but with significant differences depending on light environment. Therefore, the nursery protocol used in this study could serve as a reference for future afforestation operations for European yews planted in Mediterranean areas, with a cultivation period of two years, not being possible until now to produce high quality seedlings in one year. Other authors have recommended a cultivation period of 2-3 years using large containers (>500 ml) (García-Martí, 2007; Navarro *et al.,* 2008) for European yew planted under Mediterranean conditions.

Species with Eurosiberian distribution have not developed strategies to cope with prolonged dry periods as do Mediterranean tree species (Iszkulo & Boratynski, 2004). Nevertheless, some Eurosiberian trees, like European yew, live in Mediterranean high mountains. Seedling survival and growth in forest Mediterranean plantations depends on biomass production (photosynthesis and remobilization), and keeping water and nutrient economy during their early growth stages (Villar-Salvador *et al*., 2012). Container size, cultivation density, fertilization regimes and culture duration need to be tuned to achieve seedlings with functional attributes that maximize post-transplanting growth and yield after plantation (van den Driessche, 1991; Puértolas *et al*., 2003; Oliet *et al*., 2009).

Nitrogen fertilization has a strong influence on seedling out-planting performance in Mediterranean evergreen species (Villar-Salvador *et al.,* 2004; Villar-Salvador *et al.,* 2012). However, our fertilization treatments did not affect survival, but increased seedling growth (height and root collar diameter increased with fertilization) in agreement with results for other Mediterranean and more humid environments (Larsen *et al.,* 1988; Villar-Salvador *et al.,* 2012). Contrary to other studies on yew in Central Europe (Iszkulo, 2010), fertilization did not reduce survival. Initial seedling size was not related to survival (data not shown). Positive relationships between survival and initial seedling size and nutrient status are frequently observed in Mediterranean areas (Villar-Salvador *et al*., 2012), where vigorous initial root growth (driven by high carbon assimilation capacity) is needed during the humid season after planting to reach deep and wetter soil layers prior to the onset of the drought period (Padilla & Pugnaire, 2007). The lack of relationship between post-planting performance (survival and growth) and pre-planting seedling attributes (seedling size, RGC and nutrient status) is typical of planting sites with mild water stress conditions (Navarro *et al.,* 2006).

Most post-transplanting mortality occurred in the first summer, in contrast with Central Europe, where most of the mortality of yew is observed in winter because of the lower frost tolerance of seedlings compared to adult plants (Iszkulo, 2010). This agrees with other studies reporting that the greatest limitation to yew establishment in Mediterranean mountains survival is low soil water (Sanz *et al*., 2009; Linares, 2012). However, the higher survival at the low altitude plot and the absence of relationship between initial seedling size and nutrient status may indicate that wind or other specific site factors may play an important role in explaining plantation success in the yew plantations within Mediterranean mountain sites. As described above, the optimal environmental conditions during the first growing period after planting is essential for seedling establishment. Strong wind during the period after planting can shorten the period with optimum environmental conditions, cancelling any potential advantage of large, high N seedlings, especially in the top plane hill like site 2 (Alto de los Pájaros).

Previous studies showed that low light intensity has a negative effect on yew survival (Iszkulo & Boratyński, 2006; Iszkulo, 2010; Linares, 2012). Iszkuło & Boratyński (2004) found that survival decreased when seedlings of *Taxus baccata* had 25 cm of height when living under a conifer forest canopy. A greater survival under closed canopy could offer a competitive advantage over other forest trees, as has been shown with

Abies alba (Iszkulo *et al*., 2012). However, Thomas (2010) considered that yew maintains a high photosynthetic level under different light conditions, and Perrin & Mitchell (2013) did not find higher mortality under low light levels than under high light levels. In our study, low light had a positive effect on survival, especially in the top hill site, where only seedlings planted under closed canopy (5% PPFD) survived. It has been shown that the optimal light levels for Eurosiberian species decreases as water availability increases. For example, *Ilex aquifolium* is a facultative understory tree in most of its distribution range, but an obligate understory shrub in the driest sites of its range (Valladares *et al.,* 2005). A key adaptation to Mediterranean climate is the ability to cope with the combined effect of high light and water stress. As Eurosiberian trees are not as well adapted to those conditions (Aranda *et al.*, 2008), shade is needed during the establishment phase to avoid radiation leaf damage due to excess of light and temperature in combination with water stress.

By contrast, high light intensity at a later stage of growth increased stem diameter and height growth, in agreement with other studies (Iszkulo *et al*., 2007; Iszkulo, 2010; Perrin & Mitchell, 2013; Linares, 2012). This suggests that after seedling establishment and plants are able to access to deep soil water in summer, low light limit the photosynthetic capacity of plants (Calama *et al.*, 2013) and consequently its growth. Therefore, although initial yew initial plantation survival is maximized under close pine stands in Mediterranean mountain environments, stand thinning would be required to increase light availability in later restoration stages. An alternative to planting under dense stands could be the use of tube tree shelters, which reduce light availability and protect against wind and browsers (García & Obeso, 2003; Puértolas *et al.*, 2010). Light reduction by tree-shelter is a key factor explaining the beneficial effect of tree shelters under Mediterranean environments (Puertolas *et al.*, 2010).

A cost-benefit economic study is recommended to assess the different alternatives outlined above (Puértolas *et al*., 2012). In this sense, a great advantage of conservation or restoration of yew populations relies on its limited range, with small and low density populations located in low stress sites (Navarro *et al.,* 2013). This allows carrying out intensive post-planting management such as thinning, use of tube tree shelters and irrigation than large and dense plantations. Therefore a future challenge is to assess the short and long term effect of canopy opening or the effect of using tree shelters and their latter removal on saplings performance (Perrin & Mitchell, 2013).

In summary, cultivation of yew seedlings in 300 ml forest container for the first two years after seeding, with N fertilization of 140 and 170 mg per seedling during first and second year respectively, produces seedling of high quality for plantations in the Mediterranean mountains. Light and presumably altitude or exposures were more relevant than seedling functional attributes for explaining post-planting survival. However, seedling functional traits explained growth differences, with high-fertilized seedlings having greater growth than moderate-fertilized plants. We recommend planting European yew at moderate altitudes under a closed canopy to maximize survival. Further economic studies should be taken to determine the cost and benefit of thinning and thus having a whole perspective of the costs of yew restoration.

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