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Influence of initial light intensity and deer browsing on *Taxus baccata* saplings: a six years field study

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Abstract: European yew (*Taxus baccata* L.) is a rare species, but is the focus of great interest because of the species recovery attempts and its use in medicine. The aim of this study was to determine the optimum conditions for the development of the young generation of this species. Four-year old yew seedlings were grown under artificial light reduction: 2, 8, and 30%, as well as in full light. The seedlings were then planted in natural conditions in fenced and unfenced areas. The results of six years field studies indicated that the greatest impact on survival was shown by the light conditions from the period before planting under the canopy of trees. Seedlings from the 2 and 8% of light showed the greatest mortality. The reason was the sudden change in light conditions, because at the time of planting (early spring), there was no reduction of light under deciduous trees. Almost all of the unfenced yew seedlings were grazed by deer, but yew survival in the unfenced group was surprisingly high despite the permanent grazing. This was explained by the high resistance of yew to cutting and the protection of seedlings by a dense layer of *Rubus*. The results indicated that yew trees require protection against animals for normal development, even if animals have not previously had contact with *Taxus* and that yew seedlings are very sensitive to sudden changes in light. Both conclusions can be applied in the development of natural and artificial regeneration of European yew and other endangered plants.

Additional key words: yew, endangered species, light intensity

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Introduction

European yew (*Taxus baccata* L.) is a rare and threatened species in a major part of geographical areas (Schirone et al. 2010; Trober and Ballian 2011). Reasons for this situation are: long term human impacts (Thomas and Polwart 2003), a weak competitive ability (Iszkuło et al. 2012), and dioecy (Cedro and Iszkuło 2012; Iszkuło et al. 2011). Nevertheless, the genus *Taxus* (including *T. baccata*) is widely used in pharmacy, as taxanes are found in the needles and bark, which are used to produce a very effective anti-cancer medicine (Wani et al. 1971; Guenard et al. 1993; Iszkuło et al. 2013).

Threats and interest in the pharmaceutical industry are the reason for conservation and restoration programs of yew in many regions of the world (Smith and Cameron 2002; Zhang and Ru 2010; Farris et al. 2012; Katsavou and Ganatsas 2012). European yew is a shade-tolerant species, and although it often grows well in the full sun (Thomas and Polwart 2003; Perrin and Mitchell 2013). However, full sun negatively affects the survival of one-year seedlings in the central Europe climate, so it is suggested to grow this species in the shade (Iszkuło 2010). Nevertheless, little is known so far about the demands and cultivation of this species, especially in the early stages of development. We have no information on the influence of initial shading on the further growth of seedlings. The next most important problem is the impact of animals on European yew populations. In most yew populations in Europe a negative effect of deer on natural regeneration is observed. *Taxus baccata* is poisonous, nevertheless intense deer browsing is often observed in many places (Mysterud and Østbye 1995; Perrin et al. 2006; Farris and Filigheddu 2008). However, there are no reports which have analysed the impacts of animals which have never encountered *Taxus baccata* before. Therefore, the following research hypotheses were formulated: 1) the initial shading of seedlings affects the growth, survival and further development of *Taxus baccata*; 2) protection against animals is necessary for good growth and development of the yew.

Materials and methods

Seeds for this study were gathered from 10 individuals in the Malinówka nature reserve (south east Poland) and were prepared for germination according to methods described by Suszka (1985). In 2002, germinated seeds were sown in a nursery (geographic coordinates: N 52°14'40", E 17°06'04", 77 m a.s.l.) under similar light conditions (60% of PPFD). In 2003, germinated seeds were planted in 4-litre plastic pots. Pots were put into an artificial shading outdoor garden at 2, 8, 30 and 100% full solar radiation. Two metre-high scaffolding was covered with one or two layers of neutral density woven propylene shade cloth (Agrotex Inc.). The actual degree of light reduction under the screen was confirmed by the simultaneous measurements of photosynthetic active radiation (PAR) inside the screen and within a nearby open area. The Line Quantum Sensors (Apogee Inc.) were used to measure PAR.

In April 2006, four year-old yew seedlings were planted in 24 × 24 m plots (0.056 ha) in a 3 × 3 m spacing. Plots were established in the forest managed by Babki Forest District localized near Kórnik, Września Plateau, Poland (geographic coordinates: N 52°16'43", E 17°04'23", 95 m a.s.l.). Average yearly precipitation here is 544 mm, with average yearly temperature of 8.3°C in the Kórnik meteorological station (Cedro and Iszkuło 2011).

The experiment had two treatments (fenced and unfenced) each with three replicate plots (blocks) 24 × 24 m in size, within each 64 seedlings were planted. Distance between plots was between 10 and 20 m. Plots location was mixed – fenced and unfenced plots were not contiguous each other. In each plot were four initial light levels (2, 8, 30,100% of PPFD) schematically distributed on the same scheme. A total of 384 yew trees were planted (64 × 3 blocks × 2 fenced variants).

Plots were established under the canopy of trees of two layers, upper, larch (*Larix decidua*) and, lower, oak (*Quercus robur*). Density of larches was 86 trees per hectare, average height was 35.59 (standard error 0.3934) and average diameter was 53.61 (standard error 1.869). The density of oaks was reduced before planting in order to improve the growth conditions of yew. After reducing density of oaks was 199 trees per hectare, average height was 26.40 (standard error 0.2980) and average diameter was 26.06 (standard error 0.5543).

In the first year of the experiment, in April and at the time of full development of leaves (15 July), light intensity (RPPFD) was measured for each seedling planted, according to the methodology described above (simultaneously within and outside the forest). Each year, measurements of height, survival and grazing of saplings were performed.

The multivariate approach (MANOVA, O'Brien and Kaiser 1985) to repeated measures was used to analyse the survival and height of seedlings. Percent data (seedling survival) were transformed with an arcsine function, which normalised distributions in order to satisfy the assumptions of MANOVA. Between MANOVA subjects, the T-Test was used. Within MANOVA subjects, T-Test, Wilks' Lambda, Pillai's Trace, and Hotelling-Lawley Trace were also examined and gave consistent interpretations in all cases. All data were analysed using JMP 8.0 (SAS Institute Inc.).

Results

Light measurements in the first year under the canopy of trees, showed the average RPPFD (Relative Photosynthetic Photon Light Intensity) to be 36% (standard error 0.6545) when tress were leafless (April) and 13.95% (standard error 0.1807) at the time of full development of leaves (July). The RPPFD did not differ significantly between the variants or the blocks in both analysed periods (data not shown).

Table 1. MANOVA – Repeated Measures Analysis of *T. baccata* survival against time and treatments. Initial light means 4-year growth in different light conditions before planting (see text). Bold indicates significant values

Source of variation	Test	F-Value	DF	Prob>F
Between subjects				
Fencing	F Test	0.930	1	0.001
Initial light	F Test	3.661	3	<0.0001
Fencing × initial light	F Test	0.442	3	0.110
Within subjects				
Time	F Test	15.53	5	<0.0001
Time × fencing	F Test	10.17	5	<0.0001
Time × initial light	Wilks' Lambda	0.176	15	0.052

The highest negative influence on survival was the initial light level (indicating growth over 4 years in 2, 8, 30 and 100% of PPFD light before planting under the canopy of trees) (Table 1 and Fig. 1). The lowest survival was found for seedlings growing before planting at initial light levels of 2 and 8%. Significantly higher survival was seen at 30 and 100% of PPFD (Fig. 1). This relationship was similar in the unfenced and fenced variant as demonstrated by the lack of interaction between fencing and initial light (Table 1). There was also a significant effect of fencing on survival, with the unfenced group showing a lower survival rate. The change in survival over time was also different depending on the fencing (significant interaction of time × fencing – Table 1). This was due to the rapid decline in survival in 2008 in the unfenced variant (Fig. 1). Nearly 100% of grazed seedlings were found in the unfenced group in 2008 (Fig. 2).

In contrast to survival, no significant effect of initial light on the height of yew seedlings was found. The greatest impact was fencing and initial height (Table 2), with the effect of fencing being the most evident. The growth of seedlings was low or even negative in the unfenced variant after the year 2007, unlike the fenced variant, where the annual increment was observed in all of the tested variants of initial light (Fig. 3). The absence or low increases of height in the unfenced variant were the most likely cause of the significant interaction between time and fencing, and between the initial height and light (Table 2, Fig 3). The second reason for the significant interactions between time and the initial height and

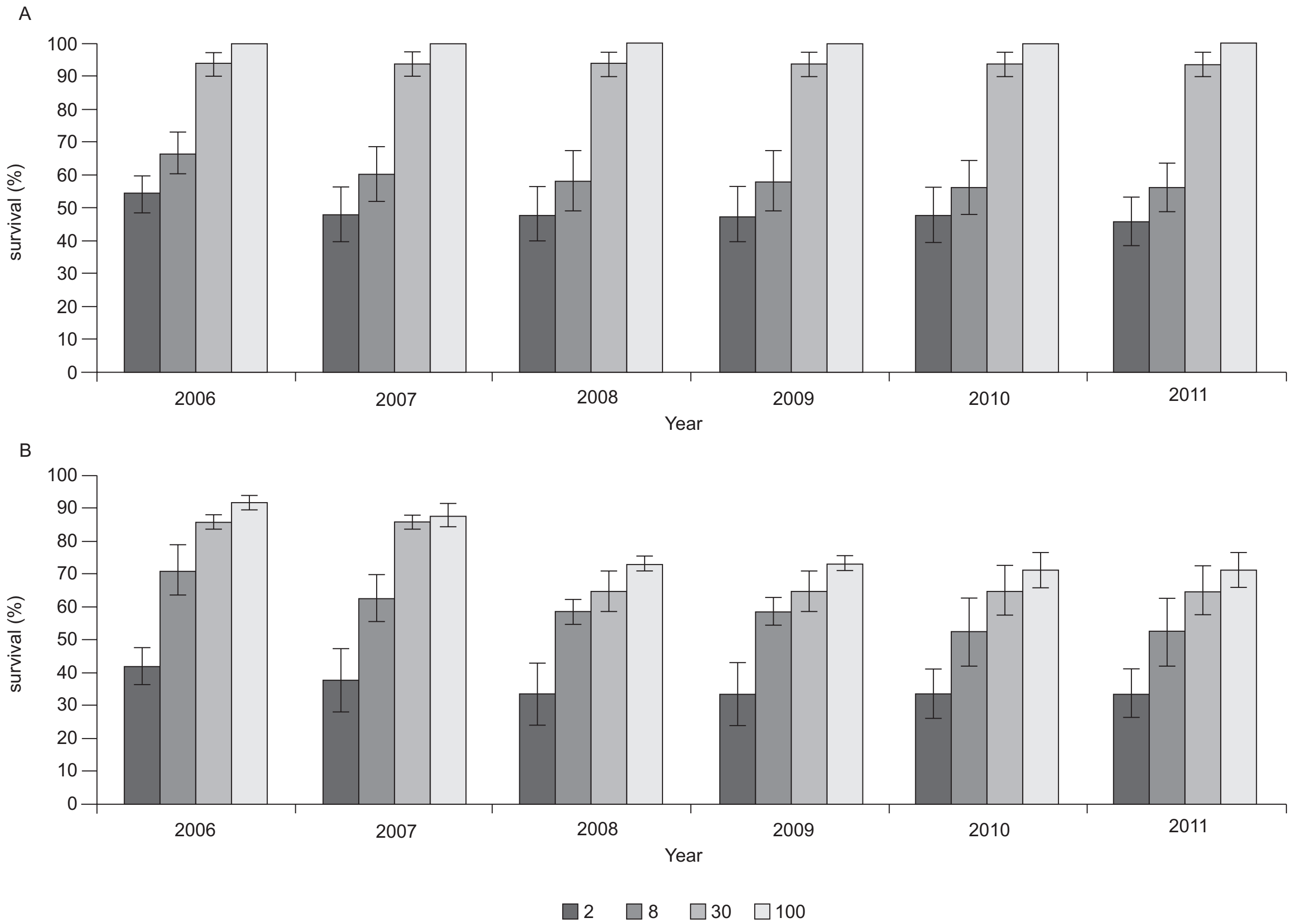

Fig. 1. The survival (± standard error) of *T. baccata* seedlings in the fenced (A) and unfenced (B) variant grown before 2006 in different light conditions (2, 8, 30 and 100% PPFD)

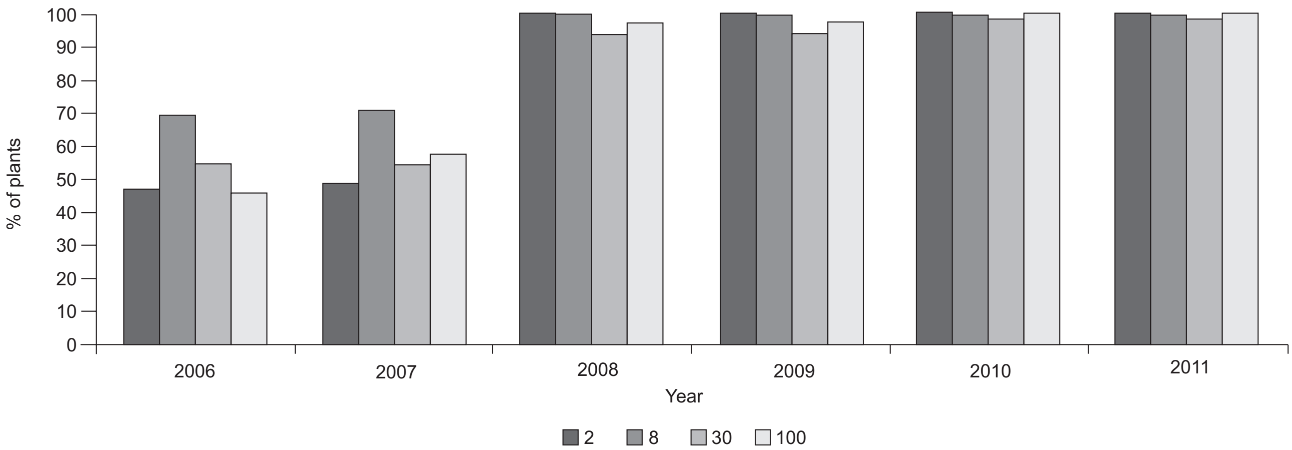

Fig. 2. Percent of grazed *T. baccata* seedlings in the unfenced variant growing before 2006 in different light conditions (2, 8, 30 and 100% PPFD)

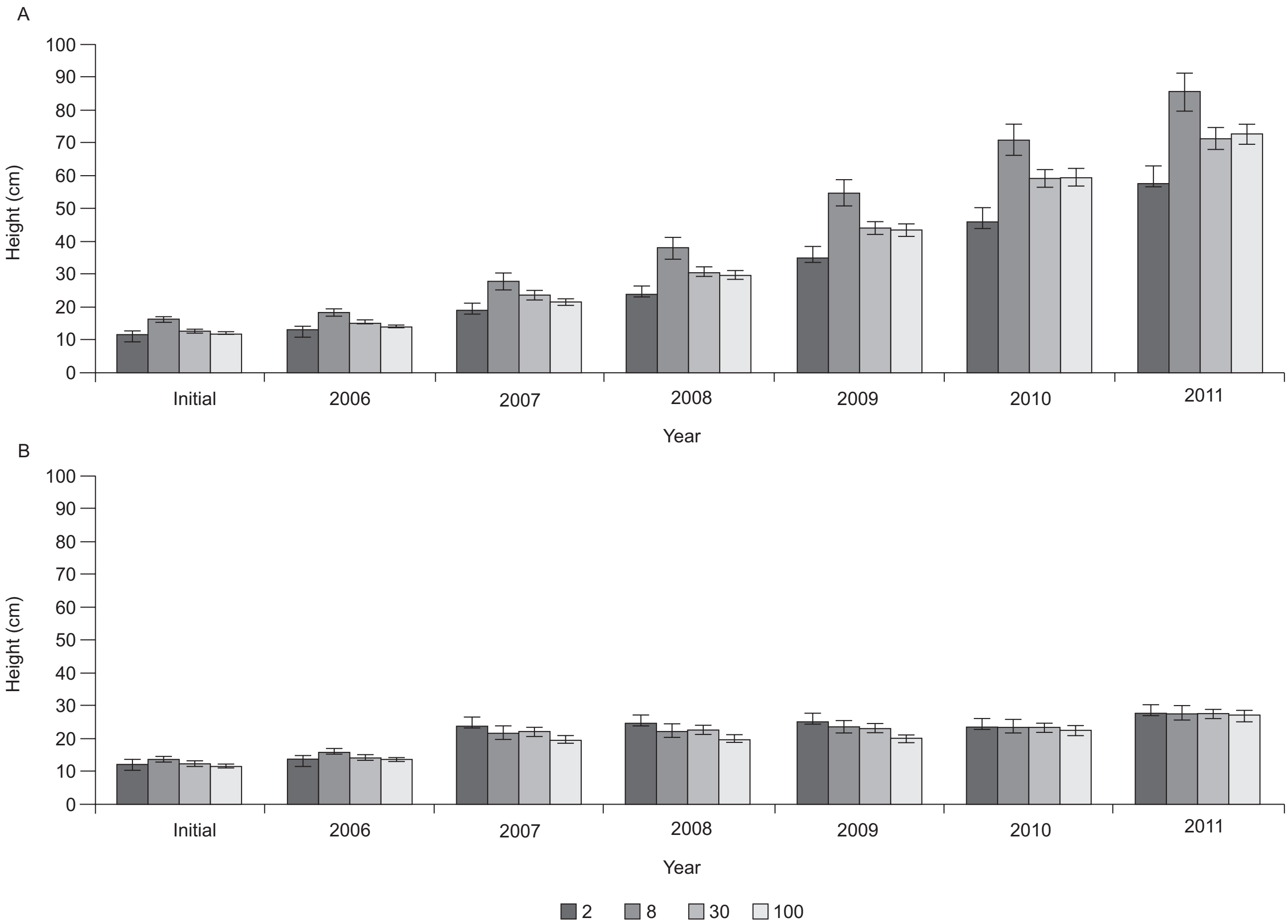

Fig. 3. Mean height (± standard error) of fenced (A) and unfenced (B) *T. baccata* seedlings growing before 2006 in different light conditions (2, 8, 30 and 100% PPFD)

light was significantly higher seedlings in the group with 8% of the initial light in the fenced variant and lack of this dominance (8%) from 2007 in the unfenced variant (Fig. 3).

Discussion

This study has demonstrated high deer pressure on *Taxus baccata* seedlings, as young trees have no chance for normal growth and development without protection. This confirms earlier reports of the nega-

Table 2. MANOVA – Repeated Measures Analysis of *T. baccata* height against time effect and treatments with initial height as a covariant. Initial light means 4-year growth in different light conditions before planting (see text)

Source of variation	Test	Value	DF	Prob>F
Between subjects				
Fencing	F Test	1.0270	1	<0.0001
Initial light	F Test	0.0274	3	0.1054
Fencing x initial light	F Test	0.0267	3	0.1133
Initial height	F Test	0.7195	1	<0.0001
Fencing × initial height	F Test	0.0680	1	0.0001
Initial light × initial height	F Test	0.0277	3	0.1030
Within subjects				
Time	F Test	0.0827	5	0.0033
Time × fencing	F Test	2.0860	5	<0.0001
Time × initial height	F Test	0.2158	5	<0.0001
Time × initial light	Wilks' Lambda	0.8917	15	0.0410

tive effects of deer on yew seedlings (Piovesan et al. 2009; Farris and Filigheddu 2008). Almost all yew trees were grazed in the unfenced variant. Nevertheless, it is surprising that their survival was relatively high (33% to 71% in the unfenced group in the last year of the experiment). Yew is a species that is resistant to cutting, and is widely used as a hedges species (Thomas and Polwart 2003). The second reason for the high survival of yew was also the dense cover of *Rubus fruticosus* agg. L., making browsing difficult below 25–28 cm. This was one of the reasons that the height of grazed seedlings did not change significantly from the third year after planting in the unfenced group. Another characteristic was the highest percentage of grazed seedlings from the group with initial light of 8% in the unfenced variant. Seedlings from 8% of PPFD were significantly higher than seedlings from other light variants and this greater exposure was probably a direct reason for the greater grazing. The positive impact of prickly plants on yew regeneration has been previously demonstrated in a Mediterranean area, where spiny shrubs mechanically protected species from browsing animals (Watt 1926; Garcia et al. 2000; Farris and Filigheddu 2008). Despite the information that shaded plants are more strongly browsed than plants growing in full sun (Baraza et al. 2010) in this study we did not find relationship between fencing (browsing) and initial light.

Initial light (before planting under canopy) had a significant influence on the survival of yew. In the first year of the experiment, the majority of plants that had been grown under 2% and 8% died out. This was not caused by the poor condition of the trees growing in this light condition, because seedlings from 8% exhibited the best parameters (survival and dry weight) (Iszkuło 2010). The direct reason for the high mortality could be a sudden change in light conditions. Seedlings were planted under the canopy of trees, but this was done in early spring, when both larch and oak were leafless (36% PPFD). Plants growing in low light develop leaves with large surface areas relative to their weight. However, at higher light intensities the area-to-mass ratio decreases, and the plant reduces the risk of an excessive temperature increase and desiccation. A similar relationship was also demonstrated in *Taxus baccata* (Wyka et al. 2008; Iszkuło 2010; Perrin and Mitchell 2013) and *Taxus brevifolia* (Mitchell 1998). Light changes also influences on the biomass allocation in plants. Seedlings growing in the shade invest primarily in above-ground part of plant. A sudden exposure to light causes higher transpiration rate and in consequently water deficit (Walters and Reich 1996; Larcher 2003). Because the most likely cause of high mortality of yew was a sudden change of lighting, it can be concluded that the rapid uncovering of seedlings can result in a significant reduction in their numbers. Therefore, the production of planting material cannot take place under too much shade (8% PPFD is too low a level of light), because the losses associated with the lack of rapid acclimation to higher light levels can be very large. For both natural and artificial regeneration, shading would result in a progressive, long-term increase in light levels.

Contrary to sudden exposure to light, sudden shading did not have negative effects for yew. *Taxus baccata* is a typical shade tolerant species (Brzeziecki and Kienast 1994) and adaptation to shade probably is easier. Shade tolerant species have also a relatively low plasticity to sudden exposure to light (Bazzaz and Carlson 1982; Valladares et al. 2002).

Despite the strong influence of light conditions before planting on survival, yew trees showed no effect of this factor on the height of trees (Table 2). However, a significant impact was seen for initial height, which was clearly illustrated by the 8% of initial light variant. This variant dominated from the first to the last years in the fenced group. Similarly, in the case of other species, an effect of initial height on annual growth has been shown (Ammer et al. 2008), which resulted in the stability of height positions in young stands (Ruha et al. 1997).

High interest of the pharmaceutical industry and programs for the protection and recovery of endangered species require the development of treatment methods for the promotion of natural regeneration and planting in natural and artificial conditions. These studies showed that yew saplings were very sensitive to light changes. In the case of regeneration under a canopy of trees, seasonal changes in light due to foliage loss should be taken into account. It is necessary to protect plants from browsing, even in areas where animals have not had previously contact with planted species.

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