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Spacing in the Nursery Seedbed and Subsequent Field Performance of *Quercus robur* L. and *Fagus sylvatica* L.

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Summary

In two nursery species important for European forests and landscape, *Quercus robur* and *Fagus sylvatica*, the impact of spacing in the seedbed on morphological characters such as height, dry weight, number of first-order-roots and first-order branches (only beech) was assessed after two-years. Height, dry weight of shoot, root, dry weight ratio between root and shoot and percentage of seedlings with six or more first-order roots were all negatively affected at lower spacing. Subsequently the effect of spacing on early field performance was determined after transplanting for two years in competition with grass and without irrigation and fertilisation simulating conditions in a forest. The initial differences between spacings in relation to dry weight were still present after two-years of transplanting. Height increment was small in oak and substantial in beech, but the initial differences in height between spacings were more or less maintained after transplanting.

Key words. *Quercus robur* – *Fagus sylvatica* – seedbed development – spacing – transplanting – growth – morphology

Introduction

European beech (*F. sylvatica* L.) and pedunculate oak (*Q. robur* L.) are prominent in the European landscape and forestry, and numerous seedlings are produced in nurseries as bare-rooted and containerised plants, where spacing is an economically issue. However, the knowledge about spacing in these two important species is limited although spacing is one of the major factors influencing seedling quality in the nursery (Van Den Driessche 1984; Aphalo and Rikala 2003). Morphological parameters such as root collar diameter, height and dry weight (DW) are altered by spacing in the seed bed (Cicek et al. 2007). Competition for light between the seedlings is probably the major cause for an effect on shoot and root growth as dry weight and number of roots are restricted by lower spacing in the field (Van Den Driessche 1984; Cicek et al. 2007) or in the containers (Aphalo and Rikala 2003). Seedling quality is crucial in transplanting success which has been correlated to morphological parameters as root collar diameter, number of roots and root and shoot ratio (Noland et al. 2001; Andersen 2004; Franco et al. 2006), and a combination of these parameters can foresee field performance better than a single one (Ziegenhagen and Kausch 1995; Noland et al. 2001), although differences exist between genotypes (Noland et al. 2001). In the present project the effect of spacing in the seedbed was studied in European beech and pedunculate oak, two species with different tolerance to shade. European beech is regarded as shade-tolerant, whereas pedunculate oak is relatively intolerant to shade (Ellenberg 1988). The hypothesis was that shade-tolerant European beech would be more tolerant to spacing than shade-intolerant pedunculate oak and the effect of seedbed spacing on the morphological characteristics of these two species and the subsequent effect on performance after transplanting for 2-years under competitive field conditions would elucidate this difference among the two species creating new knowledge to the European nursery industry.

Materials and Methods

Seedbed phase

The experiment was carried out at Aarslev, Denmark (55°18' N, 10°27' E). The experimental field was a sandy loam (13.4 % clay; 2.3 % humus) formerly arable land with soil N, P, K and Mg concentrations and pH within a normal Danish agricultural standard range. The experiment was arranged in a randomised block design with the two plant species in three separate blocks: five treatments for *Q. robur* (pedunculate oak; Danish seed source) of 400, 200, 133, 100 and 50 cm^2 spacing per plant, and six treatments for *F. sylvatica* (European beech; Danish seed source) of 400, 200, 133, 100, 50 and 25 cm^2 spacing per plant (Table 1). The low spacing in beech was chosen to see the effect of spacing on branching. The treatments were set up as separate plots in each block and pre-germinated seeds were sown the last week in April 1997 by hand, using frames squaring the seedbed to the right space between the seeds. The seedbed was 1m wide and the plots varied in length (Table 1). The number of seedlings in each plot was balanced by planting where no emergence had occurred. These seedlings, plus the guard plants at each side of the seedbed, were not used for anal-

Table 1. Spacing, density, seedbed area and number of seedlings of *Quercus robur* and *Fagus sylvatica*.

Spacing	cm^2 per seedling	400	200	133	100	50	25*
Density	Seedlings m^{-2}	25	50	75	100	200	400*
Seedbed	m^2 per plot	10.4	4.5	2.7	2.1	1.0	1.0
No. of seedlings	No. per plot	260	225	205	210	200	400

**F. sylvatica* only.

ysis or subsequent transplanting. The seedbed was covered with sand (pH 7.5) and yellow net to protect against birds during the seedling phase. The net was removed at the beginning of July in the first year. The seedlings were irrigated with sprinklers daily during the germination process and once a week from germination to September (410 mm as irrigation and precipitation from May to September). There was no irrigation the second year. The seedlings were fertilised with granulated fertiliser at a total rate of 140 kg N ha^{-1}, 30 kg P ha^{-1} and 180 kg K ha^{-1} applied in four applications (June, July, August and September) each year, which is normal practice in Danish nurseries. From July until August, the pedunculate oak seedlings were sprayed frequently with sulphur (3 kg ha^{-1}) to control oak mildew (*Microsphaera alphitoides*). Beech was sprayed twice with imidacloprid (5 kg ha^{-1}) against beech aphids (*Phyllaphis fagi* L.), while weeds were controlled by hand. In September the first year (1997), the seedlings were undercut with a reciprocating tractor-driven blade 15–17 cm below soil level as normal practice in Danish nurseries. The seedlings were lifted in December the second year (1998) by machine at a depth of app. 30 cm and placed in three-layer paper bags at −1 °C until transplanting the following spring in accordance with the normal procedure in Danish nurseries. Seedlings with scars or with two tops were excluded from further experiments.

Field performance

Ninety seedlings from each treatment and each species (30 seedlings per plot and 3 blocks) were transplanted into the field (Aarslev) in rows in a randomised block design in April 1999. The distance between the seedlings in the row was 40 cm. The planting row (30 cm) was kept free of grass and weed by hand. A mixture (12:1 g g^{-1}) of *Festuca rubra* 'Tamara' and *Agrostis castellana* 'Highland' was sown in a 60 cm wide band (20 g seed m^{-2}) between the plant rows after planting. The plots were neither irrigated nor fertilised, hence the grass competes with the seedlings for water and nutrients which reveals differences in seedling quality not observed under standard conditions (ANDERSEN 2001). The grass was cut once a week during the growing season and the cuttings were removed from the experimental area. The seedlings were lifted by machine in December 2000 at a depth of 40 cm after two growing seasons.

Climatic conditions

Precipitation and temperature, air and soil, were recorded at a meteorological station 200 m from the experimental field. Evapotranspiration was calculated from global radiation using a modified Penman equation (MIKKELSEN and OLESEN 1991).

Plant analysis

From seedlings lifted in 1998 samples of 60 from each treatment (20 seedlings per block) were used to quantify shoot and root growth. The roots were washed carefully. The percentage of seedlings with six or more first-order roots from the tap root was determined. In beech, the number of first-order branches longer than 1 cm originating from the main stem was determined, too. Height of the shoot was measured from shoot tip to the scar. Dry weight of shoot and root was determined after 48 h of drying at 70 °C. At the final lifting in 2000, 30 plants (10 plants per plot) were used for final determination of height and DW of root and shoot, as described above.

Statistical analysis

Variance procedures and comparisons of means were analysed to evaluate the effect of treatments (GLM procedure of SAS® Inc. Cary, NC, USA). Treatments were considered as fixed effects. The Student-Newman-Keuls' test was used for evaluating treatment effects, and probability values of $P<0.05$ were considered significant. Residuals were tested for normality with univariate analyses (SAS® Inc. Cary, NC, USA).

Results

2-year seedling growth

Spacing had a major effect on all morphological parameters. In both species height, DW and number of first-order roots were all negatively affected by limitations in space. Seedling height was significantly reduced in oak by 15 % and in beech by 20 %, when space was lowered from 400 to 50 cm^2 (Table 3). A pronounced effect was found on seedling DW, as 50 cm^2 reduced root DW significantly 3–4 times and shoot DW significantly 3 times compared to 400 cm^2 space, thereby lowering root to shoot ratio, with a greater effect in beech than in pedunculate oak (Table 3).

Root morphology was affected by spacing in both species. The number of seedlings with six or more first-order lateral roots from the taproot was reduced with pedunculate oak being mostly affected by limitations in space (Table 4). At 200 cm^2 the percentage of seedlings with six or more first-order roots was halved compared to that at 400 cm^2. In beech the number of seedlings with six or more first-order roots was six times lower at 50 and 25 cm^2

Table 2. Mean air and soil temperature at 10 cm depth and precipitation recorded at a meteorological station (Danish Meteorological Institute) and evapotranspiration during the transplanting period and growing season 1999 and 2000.

	Temperature (°C)		Water flux (mm)	
	Air	Soil	Precipitation	Evapotranspiration*
01.04.–30.09.1999	14.0	14.6	324	526
01.04.–30.09.2000	13.1	14.3	340	486

*Calculated according to Penman (Mikkelsen and Olesen 1991).

Table 3. Mean dry weight (DW) of root and shoot, root and shoot-ratio and height of 2-year-old seedlings (seedl.) and of 2-year-transplanted (transpl.) *Quercus robur* and *Fagus sylvatica* plants in relation to spacing (cm² plant⁻¹) in the seedbed.

Spacing	Dry weight of shoot (g)		Dry weight of root (g)		Ratio root:shoot dry weight		Height (cm)	
	Seedl.	Transpl.	Seedl.	Transpl.	Seedl.	Transpl.	Seedl.	Transpl.
Q. robur								
400	18.8 a+	47.3 a	23.2 a	89.3 a	1.27 a	2.69 a	52 a	61 a
200	11.3 b	42.9 ab	13.9 b	87.4 ab	1.29 a	2.72 a	51 ab	56 ab
133	9.2 b	41.4 b	11.2 c	82.3 b	1.29 a	2.82 a	48 b	54 b
100	9.2 b	26.7 c	9.7 c	57.9 c	1.10 b	2.81 a	48 b	51 b
50	6.6 c	27.8 c	6.6 d	53.3 c	1.08 b	2.65 a	44 c	52 b
$LSD_{0.95}$	2.16	5.34	2.31	6.92	0.101	0.298	3.34	5.17
F. sylvatica								
400	14.6 a	69.1 a	20.5 a	103.1 a	1.57 a	1.62 ab	56 a	76 ab
200	10.8 b	53.3 b	15.7 b	84.3 b	1.57 a	1.65 a	50 b	74 b
133	8.1 c	53.1 b	11.2 c	71.5 c	1.49 a	1.45 bc	46 c	82 a
100	6.7 d	56.3 ab	9.7 d	80.7 bc	1.52 a	1.58 abc	43 c	81 ab
50	5.7 de	41.1 c	6.5 e	53.2 d	1.20 b	1.44 c	45 c	73 b
25	4.6 e	23.9 d	4.7 f	35.7 e	1.05 c	1.54 abc	45 c	61 c
$LSD_{0.95}$	1.42	9.92	1.45	12.26	0.104	0.165	3.96	7.53

+Values within each column followed by the same letter are not statistically different ($P < 0.05$) according to the Student-Newman-Keuls' test.

compared to that at 400 cm². Regeneration of roots from the undercut tap-root in pedunculate oak was furthermore suppressed at low spacing (50 cm², data not shown). Spacing had an effect on number of first-order branches in beech, which was significantly lower at 133 cm² and spacing below, compared to 400 cm² (Table 4).

Field performance after 2-years of transplanting

The field performance was carried out under competitive conditions, which was intensified by the precipitation deficit, 202 mm in 1999 and 146 mm in 2000 (Table 2). The different morphological characteristics between the spacing treatments were more or less maintained after transplanting in both pedunculate oak and beech (Table 3). Height increment in oak was low (3–9 cm) in all treatments, whereas a substantial increment was seen in beech (16–38 cm) (Table 3). A significant increase in dry weight of both root and shoot was observed after the transplanting period in both species. In pedunculate oak root dry weight increased compared to shoot DW and ratio between root and shoot was doubled compared to initial value independently and not significantly between spacings. In beech the ratio between root and shoot was increased in nearly all treatments, most predominately at the lowest spacing, 50 and 25 cm², and significant differences between spacing treatments were observed after two-year of transplanting (Table 3).

Discussion

Effect of spacing on 2-year seedling

Spacing has a pronounced effect on seedling morphology reducing growth and biomass in both species probably due to changes in light quantity (Aphalo and Rikala 2006), although effect of other parameters as soil tem-

Table 4. Percentage of 2-year seedlings with six or more first-order roots in *Quercus robur* and *Fagus sylvatica* and number of branches longer than 1 cm (*F. sylvatica*) in relation to spacing (cm² per plant) in the seedbed.

Spacing	Six or more first-order roots (%)		Number of branches longer than 1 cm
	Q. robur	*F. sylvatica*	*F. sylvatica*
400	85 a⁺	30 a	8.2 a
200	30 b	25 b	7.8 a
133	25 c	10 c	7.2 ab
100	25 c	25 b	6.1 b
50	10 d	5 d	5.9 b
25	–	5 d	4.8 c

⁺Values within each column followed by the same letter are not statistically different ($P < 0.05$) according to The Student-Newman-Keuls' test.

perature and humidity in the canopy cannot be excluded. In the present experiment height in both species was significantly reduced by spacing, at 133 cm² (oak) and 200 cm² or below (beech) compared to 400 cm². Similar effects of spacing were found in ash after one-year in seedbed (Cicek et al. 2007), whereas opposite results were found in silver-birch (Aphalo and Rikala 2006). Shade-intolerant species are found to improve height to avoid shade (Aphalo and Rikala 2006), but genotype reactions to light quantity and light quality are more complex. The effect of spacing on growth was expected to differ between the two species, as pedunculate oak is regarded as shade-intolerant and European beech as shade-tolerant (Ellenberg 1988; Van Hees and Clerkx 2003), however similar effect of spacing was found in the two species. A pronounced effect on DW was observed in both species in accordance with results by others (Aphalo and Rikala 2003) and a change in allocation pattern from root to shoot was observed at low spacing (50 cm²), which has been found as an effect of low-light in pedunculate oak and beech, where shoot growth is favoured over root growth (Welander and Ottoson 2000; Ammer 2003; Van Hees and Clerkx 2003; Lockhart et al. 2008). Spacing had a pronounced influence on root morphology, as number of first-order lateral roots decreased at low spacing in agreement with results found in other species (Cicek et al. 2007). Furthermore, regeneration of roots from the undercut tap-root was suppressed at low spacing (50 cm²), supporting that limits in carbohydrates could be the main factor controlling root growth at low spacing. The negative effect of low spacing (50 cm²) on branching revealed that beech might react to changes in light quality or internal competition within the branches in accordance with results by Ammer (2003) and in silver birch (Aphalo and Rikala 2006).

Effect of initial spacing on field performance

In Danish nurseries, pedunculate oak and beech is usually field cultivated at spacing around 133 cm², in order to diminish field costs. The average size of seedlings at 400 and 200 cm² in the present experiment would therefore be larger than in a typical nursery production. The present results show a positive effect on DW, but not on height increment of using larger seedlings, concurring with results of Noland et al. (2001). The morphological differences in DW between the spacing-treatments were more or less maintained after two-year of transplanting in both pedunculate oak and beech, confirming the importance of initial seedling morphology in relation to early field performance (Noland et al. 2001; Aphalo and Rikala 2003).

The differences in height were more or less maintained after two-years of transplanting in accordance with observations by Ammer et al. (2008), who found height increment in beech determined by previous-year height. The generally low height increment in the present experiment was probably caused by the severe competition between the seedlings and the grass as observed in earlier experiments (Andersen 2004). The initial differences in root and shoot ratio between the spacings in both species vanished revealing a plasticity in root growth as found by Meier and Leuschner (2008), probably as a reaction to the high precipitation deficit and competition from the grass. In pedunculate oak root growth was enlarged after transplanting especially at the seedlings from the lowest spacings and consequently root to shoot ratio evened out in accordance with results in northern red oak under drought stress (Jacobs et al. 2005) and in consequences of competitive conditions as in sessile oak (Jakobs et al. 2009). This emphasizes the importance of an initial high root-shoot ratio for re-growth in temperate climate with major weed competition according to Martens et al. (2007). No specific effect from the number of first-order lateral roots could be elucidated in the present experiment in accordance with results by Jakobs et al. (2005). Field performance of the seedlings in relation to spacing didn't reveal differences between the two species in relation to pedunculate oak being shade-intolerant, and beech being shade-tolerant. In both species nursery spacing was crucial in relation to field performance even after two-years and spacing should be given attention in the nurseries independently of genotype.

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