

## Ex-vitro growth studies of *Quercus robur* L.

Seán Mac An tSaoir<sup>1</sup> and Jennifer O'Brien<sup>2</sup>

### Abstract

Micropropagated oak trees (*Quercus robur* L.) were established in a replicated field trial together with conventional seedlings at Castlewellan Forest Park, Co Down. At the end of the first year (1994) the micropropagated trees were significantly smaller than the seedlings (49 v 65 cm). Five years later there was no significant height difference between the two planting stock types (218 and 220 cm respectively). No abnormal growth was observed in any micropropagated trees during the course of the experiment.

**Keywords:** *Quercus robur* L., micropropagation, *in-vitro*, *ex-vitro*.

### Introduction

*Quercus robur* L. is one of the main forest species in the British Isles. Tree improvement in the species is difficult, due to its slow maturation and marked fluctuations in acorn production (Carmen *et al.* 1987). Consequently there is a considerable interest in short-circuiting traditional improvement methods by identifying elite oaks in the forest and propagating them vegetatively. Unfortunately the potential of *Q. robur* cuttings to form adventitious roots decreases rapidly with increasing plant age (Chalupa 1993). However, vegetative reproduction in the genus *Quercus* can occur through stump and rhizomatous sprouting (Muller 1951). During COST<sup>3</sup> Action 87 a propagation system was developed whereby *in-vitro* material was successfully established from adult trees, thus allowing the genotype to be micro-propagated (Evers *et al.* 1990). It was necessary, however, to examine the growth performance of the micro-propagated planting stock following field planting, in order to determine if there were *in-vitro* effects leading to abnormal growth.

### Materials and methods

The *in-vitro* clone (NL 100) was established in culture from sprouted shoots supplied in the autumn of 1991. These were from trunk segments of a 100-year old *Q. robur* tree, which had been harvested in the forest of Oosterengh, near Wageningen, The Netherlands (Evers *et al.* 1995). Acorns were subsequently gathered from the same stand of trees in 1991.

<sup>1</sup> Corresponding author: Department of Applied Plant Science, Northern Ireland Horticultural and Plant Breeding Station, Department of Agriculture and Rural Development for Northern Ireland, Loughgall, Co Armagh (sean.macantsaoir@dardni.gov.uk).

<sup>2</sup> Department of Veterinary Science, Department of Agriculture and Rural Development for Northern Ireland, Stoney Rd, Belfast.

<sup>3</sup> COST: European Co-operation in the Field of Scientific and Technical Research <http://www.belspo.be/cost/>



The clone was micro-propagated in Woody plant media (WPM) (Lloyd and McCown 1980) with 0.2 ppm BAP as described by Chalupa (1984). The shoots were rooted in 1/3 WPM containing 0.3 ppm IBA, 20 g l<sup>-1</sup> sucrose, solidified with 7 g Difco Bacto agar. During the multiplication stage the shoots were grown under one of three different light regimes – Grolux (high red light component at  $\lambda$  660 nm), Cool White (half the red light of Grolux at  $\lambda$  660nm but twice the blue light at  $\lambda$  430 nm) or a combination of both (two light tubes of each type as opposed to four tubes of one type). The stimulating effect of red light on the micropropagation of oak has already been reported (Mac An tSaoir and Kabrianis 1993). In the spring of 1992 the plantlets were established *ex-vitro* in a glasshouse with seedlings which had been germinated from the acorns sown in trays of sand during the winter of 1991/92. Thus the two planting stock types were of a similar age. In 1993 all plants were moved outside the glasshouse and grown-on in pots.

The following spring (1994) the seedlings and micro-propagated plants were planted in a field trial at Castlewellan Forest Park, Co Down, at 140 m elevation (Irish Grid reference: J 333364). The micro-propagated plants and seedlings were planted in alternate plots of 20 trees. Each plot measured 8 x 8 m with a tree spacing of 0.8 x 2.0 m. There were four rows of five oak trees in each plot (with a beech tree planted between each pair of oaks). The treatments were replicated five times in alternate blocks. The height of each tree was recorded at the end of each growing season and plot means calculated. The means were then used to compute an analysis of variance.

The heights of the micro-propagated trees were further analysed to determine if there was any carryover effect of the light treatments *ex-vitro* (each micro-propagated tree had been labelled *in-vitro* according to the light treatment it had been subjected to).

## Results

In the first year plants grew taller when grown under the Grolux light regime than in the Cool White or combined treatment (Table 1). By the second year when the plants were moved *ex-vitro* there was no significant difference between any of the light treatments.

**Table 1.** *The effect of in-vitro light quality on tree height under glasshouse conditions.*

Year	Light treatment			Significance	Standard Error
	Cool White	Grolux	Cool White/ Grolux		
		cm			
1992	5.53	7.70	5.87	p≤0.001	0.358
1993	17.83	20.60	18.90	ns <sup>1</sup>	0.924

<sup>1</sup> Differences between means not statistically significant

While the micropropagated material was considerably smaller (17.5 cm or 2.73 times) than the seedlings at the start of the experiment (Table 2), this difference gradually decreased over time. By the end of the first growing season in the field the difference was no longer statistically significant.

Abnormal growth patterns were not observed in the micropropagated material during the course of the experiment.



**Table 2.** *Effect of propagation method on the mean height of Q robur micro-propagated (C) plants and seedlings (S).*

Year	1992	1993	1994	1995	1996	1997	1998	1999
	cm							
C	6.4	19.1	48.8	87.8	116.6	163.6	183.0	218.0
S	23.9	47.9	64.8	96.4	127.0	169.2	189.0	220.4
Significance	p<0.001	p<0.001	p<0.001	ns <sup>1</sup>	ns	ns	ns	ns
Standard error	0.84	1.27	1.40	2.59	3.37	4.49	5.92	8.39

<sup>1</sup> Differences between means not statistically significant

## Discussion

Several laboratories in the COST group failed to establish field trials as the cultures of the NL 100 clones they received did not propagate very well. It subsequently transpired that the clones which were distributed to the different research partner organisations were derived from a range of trunk sprouts (the positions of origin of which had not been recorded). This raises the possibility that some vegetative shoots were more juvenile than others were. Thus, if a laboratory received material established from a lower (and therefore potentially more juvenile) trunk segment, propagation may have been easier in comparison to those who received material from further up the trunk.

Given that predominantly red light emitting tubes (Grolux) significantly increased the shoot multiplication rate over predominantly blue light emitting tubes (Cool White) *in-vitro*, there was always the risk that the plants grown under the former light regime would develop into bushier trees *ex-vitro*. However, the data show that while there was a significant height difference between the treatments at the end of the first growing season; by the end of the second year this difference was no longer significant.

The data show that the system developed by Evers *et al* (1990) can be used to propagate oak material *in-vitro* and that the clones grow normally when compared to seedlings of the same age. Given the food reserves that are present in acorns it was expected that the seedlings would initially grow faster than the clones. This proved to be the case. However, as the data clearly show (Table 2), this height difference quickly disappeared. Since the clone was selected from a proven elite tree and the acorns were of mixed genetic origin (albeit from a superior stand), it might be expected that the clone would demonstrate superior growth, as shown by the results (Table 2).

Comparison with data recorded between 1994 and 1997 from other field trials, which used the same genetic material, shows that the results presented here are typical. In two of the field trials where the clones were taller than the seedlings at establishment, the former remained taller (Hammatt and Jones 1997, Monney and Schmid 1998). In three other trials where the seedlings were significantly taller at the start, the difference persisted (Hunter and Moore 1998, Appelgren 1998, Wilhelm and Cachée 1998). However, the increment after planting for both stock types remained about the same. This gradually reduced the relative height difference between the two and its statistical significance. It is reasonable to conclude that in this instance planting stock produced from tissue culture, derived from vegetative juvenile sprouts removed from adult trees, grew normally *ex-vitro* and at the same rate as seedlings five years after planting.



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