Vegetative Propagation of Oak – What are the Best Options?

A Literature Review and Contacts with Practitioners

Richard Worrell

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Contents

Summary

The aim of this report is to review recent progress in the vegetative propagation of *Q. robur* and *Q. petraea*, via cuttings and micro-propagation, in order to determine whether cost effective methods are available. The project combined a literature review (of papers mainly publish post-1995), web searches, and interviews with key practitioners.

Cuttings appear to be the only potentially viable technique for plant production in large numbers, and experience from Germany suggest that this might be feasible on a commercial or semi-commercial basis. In contrast, micropropagation systems for *Q. petraea* and *Q. robur* appear only to have been used for research purposes, rather than in any form of commercial production. So the main conclusion of this review is that FTT should consider developing cuttings expertise based on the German experience.

The process for plant production could be:

1. To collect acorns from the BSOs as soon as they start fruiting and use these to produce stock hedges.
2. For clones where acorns were particularly scarce, it might be sensible to multiply these up by micropropagation. This would depend on whether if this was feasible at reasonable cost - the advantage being that acorns could be multiplied up more rapidly by micro-prop than by using cuttings techniques, and this could shorten the project time scales.
3. Develop cuttings expertise and nursery protocols with the full involvement of FTT nurseries.
4. Make the stock plants available to nurseries for propagation.

Cuttings: Literature Review

Cutting techniques have been used commercially to multiply up small numbers of valuable oak cultivars for sale; and there are two reports of cuttings production at larger scale (tens of thousands of plants) in forestry in Germany during the 1980-90s. This work was discontinued when use of improved material was discouraged by German forest policy. There was a small and short-lived Forest Research programme at Alice Holt involving propagating oaks by cuttings from coppice shoots.

The potential of cuttings as propagation method is hard to evaluate accurately from a literature search alone, because of somewhat contradictory messages over the decades. Researchers involved primarily in tissue culture appear to dismiss cuttings as an approach; whereas practitioners involved in trialling cuttings generally report success. Propagation using best practice for softwood cuttings from young plants or stock hedges typically gives rooting percentages between 35% and 80%; though it should be noted that these results are mainly from carefully conducted research trials, and the higher success rates (>60%) might not necessarily be achievable in commercial practice. The rooting percentage in the mass trials in Germany were 59%; and these plants typically reached lengths of 30-50 cm by the autumn and overwintered with only small losses. They were then transplanted to a nursery with 78-94% survival, and went on to produce vigorous plants.

1-3-year-old trees can be made to root at high frequencies and produced well-developed root systems. The use of cuttings from very young plants is limited however, because the quantity of cutting material produced by young ortet is low; though this can be overcome to some extent by hedging. Trails with hedged plants 3-9 years old (of *Q. robur*) showed high success i.e. 81% of

cuttings rooted. Successful cutting propagation requires rooting under the continuously high humidity of a fog system. Long term performance of plants from cuttings appears to be at least as good as use of seedlings.

Propagating proven physiological mature genotypes is not feasible using cuttings taken directly from mature trees. Instead the best options would appear to be to:

- use seedlings grown from acorns produced by plus trees, arresting onset of physiological maturity by hedging, then conducting long-term validation trials of the mother trees.
- grow a small number of plants from forced epicormics shoots from branch sections from known good genotypes, say by micro-propagation, then multiply these via stock hedges and cuttings.

Many authors supply estimates of rooting percentage of cuttings (the number of cuttings that produce roots), but fewer provide data on the quality of the roots, or whether the roots are adequate to produce a viable plant. Even fewer provide data on plant survival and subsequent growth. There is very little data on costs of production.

Cuttings: Contact with practitioners

Two practitioners were contacted: Prof. Wolfgang Spethmann of University of Hanover (retired) and Prof Daniel Struve Prof Daniel Struve of Ohio State University. Wolfgang Spethmann stated that the oak improvement programme in Germany followed a similar route to that currently being progressed by FTT i.e. plus tree selection, seed orchards followed by development vegetative propagation by cuttings method. Vegetative propagation by cuttings was used successfully during the 1980s and 1990s; with 5-8 state forest nurseries with each producing 20,000 to 40,000 plants per year. Spethmann states that oak cuttings are easy to root. The main parameters important for success are: using a setting date in the middle to end of June when the leaves are still light green; and stock plant age limited to 7 years (before cutting back again). Use of a high pressure fog system that maintains humidity (> 90 % all the time until September) is also important. The price of conventional cuttings was approximately 3 times that of seedlings. Daniel Struve confirmed that his more limited experience of working with oak cuttings suggested that producing plants via cuttings is relatively straightforward and has considerable potential.

Micropropagation: Literature review

Micropropagation systems have been developed for *Q. petraea* and *Q. robur*, but appear only to have been used for research purposes, rather than in any form of commercial production. We were only able to find one commercial company with experience of oak micropropagation in the UK, and their view was that it was not commercially viable, and they were not interested in further work. However micro-propagation could still be of potential use; for example for quickly multiplying up particularly rare acorns to produce plants for stock hedges.

There appear to be reproducible protocols, but outcomes are still subject to considerable variation in success due to the effect of genotype (differing success rates with individual trees). The protocols appears to be effective, even with adult material, but techniques are not sufficiently cost-effective to be applied on a commercial scale. Note that work on “conventional micro propagation” continued with *Q. petraea* and *Q. robur* until about 2000, after which the research focus for these two species largely switched to somatic embryogenesis.

Protocols are based on in vitro multiplication of shoots from axillary buds. The easiest way of developing cultures is using embryos from acorns (=zygotic embryos) and seedlings as explants. However progress has also been made in using tissues from mature trees, which is important because this potentially allows trees of known good phenotype or genotype to be propagated. The recommended approach when using material from adult field trees as explant sources is to use greenhouse /growth chamber forced sprouts from cut branches, instead of field-collected material.

Problems of bacterial contamination of cultures and release of toxic mainly phenolic compounds into the growth medium have both largely been overcome. Declines in the proliferation capacity of oak cultures after several subcultures is a common bottleneck. It appears to be possible to overcome this by placing shoots horizontally on the medium (explants are traditionally orientated vertically) and transferring these onto fresh medium after 2 weeks.

Rooting of microshoots can be achieved either in vitro, or ex vitro under nonsterile conditions by direct rooting in potting mixture. Rooting of plantlets remains something of a bottleneck, with variable success. Ex vitro rooting is less laborious than in vitro rooting. Acclimatisation of plantlets involves growing them under high humidity for several weeks, then gradually reducing humidity to normal levels. Field growth of micropropagated oak trees is comparable to that of control seedlings.

It is hard to summarise success rates because relatively few studies follow the process right through to field grown plants. Rooting percentages of 60-90% are recorded when using explants of juvenile origin; and whilst rather lower success rates are generally reported for adult-origin explants, some studies have achieved nearly the same success levels. There is strikingly little information published on costs and commercial potential of producing oak by micropropagation. Specific oak cultivars for sale at high unit process (£10-70 per plant dependent on size) are reported on the web, though it is not always clear if these have been vegetatively produced.

Micropropagation: contact with practitioners

The following commercial micropropagation businesses were contact: Gentech Propagation Ltd, Dundee (Nigel Ebblewaite) and Micropropagation Services, Loughborough (Barbara Wright). Gentech stated that they had no expertise with Quercus; but if they could be supplied with suitable initiation material, they could attempt to initiate it into sterile culture. Micropropagation Services stated that they did do some oak micropropagation 10-15 years ago but did not find it anywhere near being an economic proposition; and that their business has changed and they would not be interested in looking at this further.

Ana Vieitez (Instituto de Investigaciones Agrobiológicas, Spain) was contacted to ask if she was aware of any commercial ventures in oak micropropagation but no response was received. We were unable to find contact details for Vladamir Chalupa.

Next steps

The proposal to develop cutting expertise should be critically assessed by the Oak Group. A first step would be for FTT to start a dialogue with field staff who were involved with the German programme and if this continues to be useful, to organise a trip to Germany.

1. Introduction

1.1 Aim and methods

The aim of this report is to review recent progress in the vegetative propagation of *Q. robur* and *Q. petraea*, especially via cuttings and micro-propagation, in order to determine whether cost effective methods are available for producing plants from selected trees for use in the nursery trade. A literature review was carried out; web searches were undertaken to try to locate any businesses or agencies that were propagating oak vegetatively; and in addition key practitioners were contacted by email.

The literature used in this study was:

- For cuttings – abstracts and papers published in the period 1985-present, from both forestry and horticulture research organisations.
- For micro-propagation – reviews dated 2010 and later, and key papers back to about 1990. The volume of published work in this field is large, especially when oaks other than *Q. petraea* and *Q. robur* are included, and some of the literature is very technical - hence the need to focus primarily on review papers.

1.2 Possible methods of propagation

The methods for vegetation potentially available for the vegetative reproduction of are:

1. Grafting – scion material from selected trees grafted on to rootstock.
2. Cuttings – rooting of shoot material from seedlings and young trees, stock hedges, stump sprouts, or grafted plants.
3. Micro-propagation – rooted micro-shoots grown in vitro from acorns / embryos, seedlings and shoots from older trees.
4. Somatic embryogenesis (SE) – plantlets grown in vitro mainly from shoot, bud and leaf tissues, with both shoot and root structures developing during culture[1].

Whilst in these methods are generally considered in isolation, some authors point to combinations of techniques as possible routes towards commercial deployment.

All these 4 methods are all difficult in *Quercus* species compared to other tree species, but because seeding in improved oak is so problematic, considerable research effort has nevertheless been put into attempting to develop vegetative propagation.

[1] The review papers on micropropagation used in this study also gave accounts of the status of SE in oak and the author has made rough notes on this, which could be made available to interested parties. SE appears to be reaching commercial applicability for cork oak, but not *Q. robur* of *Q. petraea*; though incremental progress continues to be made on these species.

2. Cuttings

2.1 Introduction

Oak stem cutting propagation protocols typically involve softwood and semi-hardwood stem cuttings collected from juvenile stock plants (with various stock plant manipulations), treated with indole butyric acid (IBA) basal dips.

Early research demonstrated that it is very difficult to root cuttings of oak, particularly if they are taken from mature trees; and that rooting ability declines quickly after about 3 years of age. However, some progress has been reported in the period 1985-2014 using cuttings from both stock hedges and young plants; with two papers (Spethmann 1986 and Spethmann and Harms 1993) claiming that this can be done commercially. There was a small and shortlived Forest Research programme at Alice Holt involving propagating oaks by cuttings taken from coppice shoots (Harmer and Baker 1991, Harmer 2010).

This report focuses on work reported during the last 30 years, to capture progress made using modern approaches such as the use of plant growth regulators and misting equipment. It should be noted that many authors supply estimates of rooting percentage of cuttings (the number of cuttings that produce roots), but fewer provide data on the quality of the roots, or whether the roots are adequate to produce a viable plant. Even fewer provide data on plant survival and subsequent growth.

2.2 Recent studies evaluating potential - *Q. robur* and Q*. petraea*

Drew and Dirr (1989) used cuttings from 3 year old stock plants of *Q. robur* and showed that this species gave the second highest rooting percentages (30-67%) of the 10 *Quercus* species tested. The authors concluded that propagation by cuttings is feasible for some species, including *Q. robur,* but not for others (Table 1).

	Rooting percentage		
Species	**May 15**	**June 18**	**Aug. 20**
Quercus alba	1 e[z]	0 d	0 e
Quercus coccinea	24 d	31 c	10 de
Quercus lyrata	43 c	46 b	40 b
Quercus palustris	20 d	20 c	6 e
Quercus phellos	80 a	76 a	64 a
Quercus robur	67 b	62 a	30 bc
Quercus rubra	19 d	69 a	2 e
Quercus shumardii	17 d	15 c	22 dc
Quercus suber	2 e	2 d	2 e
Quercus virginiana	13 d	45 b	34 bc

[z]Mean separation within columns by Duncan's New Multiple Range test, $p \geq 0.05$.

Harmer and Baker (1991) and Harmer (2010) rooted a small number of cuttings derived from coppice shoots grown from the stumps of 10 year old oaks cut back to ground level the year before. They reported that getting the cuttings to root was not difficult; and approximately 100 clonal plants were established in a small trial looking at the relationships between the growth and crown structure of the plants and their mother trees.

Griffin and Bassuk (1996) achieved very variable rooting percentages of 0-61% for *Quercus robur* using a variety of treatments to the stock plants (see below), with best success recorded using etiolated shoots. Their work demonstrates that if techniques are wrong, very low rooting percentages result.

Ferrini and Bassuk (2002) recorded average rooting percentages 76% for cuttings taken from 45 day old seedlings and 3 year old stock plants of *Q. robur* ; and the mean root number was 4.5 and root length 11cm. They applied 3 treatments (etiolation using Velcro strips, blanching and inoculation with Agrobacterium rhizogenes), all of which turned out not affect rooting percentage, but did improve rooting quality. Rooting of cuttings taken from for older plants (20 years) was far worse (see "table 8" below).

Table 8 - Effect of mother plant age on rooting percentage of *Quercus robur* cuttings (average of different treatments and different site of origin) (average of the different treatments)

Mother plant age	Rooting (%)	Roots number	Root length (cm)
Seedlings (1 month)	76.26 a	4.51 a	10.99 a
3-year-old	34.7 b	1.69 b	7.49 b
20-year-old	3.57 c	1.3 c	2.33 c
Significance level	0.000	0.037	0.000

Chalupa (1993) reported successful propagation of both *Q. robur* and *Q. petraea* (see tables I and II below) using cuttings taken from:

1. 1-3 year-old trees: these rooted at high frequencies and produced well-developed root systems. The use of cuttings from very young plants is limited however, because the quantity of cutting material which is produced by young ortet is low; though this can be overcome to some extent by hedging.
2. hedged 6-year-old plants of Q. *robur*: these showed high success i.e. 81% of cuttings rooted (c.f. 56 % from unhedged 6 year old plants).

In contrast, cuttings from older trees (9-30 yrs) rooted poorly and he concluded, in line with all previous research, that difficulties associated with aging exclude the direct use of cuttings from older trees for clonal propagation at commercial scale.

Table I. Rooting of *Quercus robur* cuttings collected from plants of different ages.

Age (yr)	*Rooting (%)*	*Mean number of roots/ rooted cutting*
1	89a	3.8a
3	78a	3.2a
7	52b	2.6ab
9	39b	2.2b
20	18c	2.0b
30	11c	1.7b

The data are based on 50 cuttings/treatment. Values followed by the same letter are not significantly different at the 5% level.

Table II. The effect of hedging of *Quercus robur* (6-yr-old plants) on rooting of cuttings and on formation of new shoots during rooting.

Stock plants	*Rooting (%)*	*Mean number of roots/ rooted cutting*	*Formation of new shoots (%)*
Control plants	56a	2.7a	7a
Hedged plants	81b	3.9b	62b

The data are based on 90 cuttings per treatment. Figures followed by the same letter are not significantly different at the 5% level.

Whilst the data in tables I and II relate to *Q. robur*, Chalupa also records rooting percentages of 76 - 92% for Q*. petraea*. For *Q. robur*, cuttings from hedged plants showed higher rooting percentages, and importantly higher frequencies of formation of new shoots, than from unhedged trees of the same age. He also recorded that rooted cuttings (of both species) that formed new shoots, typically reached lengths of 30-50 cm by the autumn and overwintered with only small losses. They were then transplanted to a nursery with 78-94% survival, and went on to produce vigorous plants.

Struve et al (2010) report that stem cuttings from seedlings from two parent *Q. petraea* trees rooted in high percentages (72-100% see "table 4" below). Cuttings were rooted successfully in both mid-June and early August with high survival percentages. Overwinter survival of cuttings from all propagation dates was 96% (303 of 314 rooted cuttings).

Table 4. Percent rooting of semihardwood cuttings from four sources of *Quercus* at two propagation dates.[z]

Species	Source	Number of Stock plants	Number of Cuttings June	Number of Cuttings August	Propagation date (rooting %) June	Propagation date (rooting %) August
Q. petraea	Baccardi	29	63	43	72 A[x]	100 A
	Caiano	15	29	17	96 A	100 A
Q. pubescens	Camaldoli	19	22	37	84 A	89 A
	PDP	27	35	44	85 A	97 A
				Average	82 A[y]	97 B

[z]Cuttings were collected from the same stock plants at each propagation date.
[y]Means for the average percent rooting for the two propagation dates are significantly different from each other at the $\alpha = 0.05$ level of significance using the Waller-Duncan mean separation test.
[x]Means within a propagation date followed by different numbers are significantly different from each other at the $\alpha = 0.05$ level of significance using the Waller-Duncan mean separation test.

Spethman (1986) reported the "first successful production of plants on a practical scale achieved in Uslar, Germany with 26,000 cuttings". Cuttings from mother trees of different ages exhibited a clear decrease in rootability with increasing age, viz. 90, 60 and 30% rooting with mother trees 2, 6 and 20 yr old respectively. Rooting started after 3-4 weeks, lignification after 5-6 weeks, and main root growth ended after 10 weeks. Rooting started without previous callus formation, and callusing was more frequently observed in sub-optimum conditions and with increasing age of mother trees.

Spethmann and Harms (1993) developed a practical mass propagation of 38,000 *Quercus robur* and *Q petraea* plants via cuttings. For this they used cuttings from hedged mother plants 3-5 years old, cut back to 5-20 cm tall every March. They were able to continue this work successfully when the stock plants reached 9 years (and presumably more) years of age. They reported a mean rooting percentage was 59% amongst their 38,000 cuttings, 19% were unrooted, 22% had formed a callus. Despite this, they give the impression of the plants being produced at reasonable costs (see below).

Hartmann et al (2010) reported some success in rooting leafy softwood cuttings of *Quercus robur* 'Fastigiata' under outdoor mist; and that long (90 cm), semi-hardwood cuttings of *Q. robur 'Fastigiata Koster'* also rooted well when treated with 5000 ppm IBA and propagated under high pressure fog systems. Rooting was enhanced and production time reduced with the larger cuttings.

Iqbal et al (2014), attempted both hardwood and softwood cuttings taken from older (5-20 year old) *Q. robur* trees in Kashmir; treated with different levels of IBA and NAA. The hardwood cuttings largely failed, though one treatment showed 30% rooting; the softwood cuttings fared slightly better, with one treatment showing 51% rooting (though note that a cutting having one root > 1mm was recorded as rooting !). This shows: a) the difficulties of working with cuttings from relatively mature trees; b) that some success can nevertheless be achieved with for auxin treated softwood cuttings.

Spethmann (2015) reported successful propagation of oak using very long cuttings (60-120 cm), grown from cut-back stock plants 9-30 years old.

Successful rooting of cuttings has also been recorded for a number of other oak species, using cut back stock plants, stump sprouts, induced adventitious sprouts or rejuvenated scion material for example :

- the Mediterranean oak *Quercus ithaburensis* (Eshed et al 1996);

- some North American oaks e.g. Amissah et al (2008), Hartmann et al (2010), Gocke and Robinson (2010);
- some Asian oaks: Mishra et al (2003), Purohit (2005), LiBin et al. (2007) and Nautiyal et al. (2013).

2.3 Methods

2.3.1 Cuttings from hedged plants

Chalupa (1995) states that hedging of oak stock plants offers an effective technique for the production of cuttings with high rooting potential and high survival. He collected cuttings from 6-year-old hedged stock plants (hedged annually 4-10 cm above the ground) between May 20 and July 20. All cuttings were inserted into the rooting mixture 2-24 h after being taken from trees. Bases of leafy cuttings (10-20 cm long) were soaked in a hormonal solution (20-24 h in indole-3-butyric acid (IBA) 200 m-)g·11 or treated with a talc based rooting powder (1% IBA + 10% benomyl or 0.5% IBA + 0.1% naphthalene acetic acid (NAA) + 10% benomyl), and inserted into rooting mixture consisting of peat and perlite (1:1 or 1:1.5, v/v). Cuttings were rooted either under controlled environment (in growth cabinets equipped with a fog system) or in a greenhouse under an intermittent fog system. After rooting, relative air humidity and temperature were gradually reduced, and rooted cuttings wintered in the rooting mixture in the same place in the unheated greenhouse. Rooted cuttings were lifted the following spring (in early June, after formation of new shoots) and were transplanted in the nursery.

Spethmann and Harms (1993) took cuttings 10-15-cm long of *Quercus petraea* and *Q. robur* from 3-5-year-old mother plants harvested during the period 1-13 June. The lower leaves were stripped and the cutting base dipped in 0.5% IBS (in talc) + 10% Euparen. The substrates were fertilised with 9 concentrations of the slow-release fertilizer Osmocote Plus (N:P:K:Mg, 15:11:13:2, 3-4 mo) and 5 concentrations of the quickly soluble Nitrophoska Spezial (12:12:17:2). The fertilizer was mixed with the upper 30cm of the substrate. In a polytunnel (10 x 20 m) one-half of the ground bed was filled with gravel (3-8 mm), the other half with a mixture of peat:sand:perlite: styromull (1:1:1:1). 650 cuttings were set in each of the 2.5 m2 plots. A total of 18,200 cuttings were planted in fertilized and 18,200 cuttings in the unfertilized substrate. A high-pressure fog system (Norrison) controlled by a hygrostat was used to maintain high air humidity and a mist system to water the substrate when necessary. There was no base heating. The greenhouse was shaded and remained closed even when the air temperature went up to 50 °C. Rooting started after 3 weeks. In September the cuttings were hardened by reducing air humidity and ventilating the greenhouse.

Iglesias-Diaz et al (2000) studied the effect of banding on the rooting of cuttings centenarian clone of *Quercus robur var. fastigiata* growing in Galicia, Spain, testing different timings of taking cuttings. Hartmann et al (2010) used leafy softwood cuttings of *Quercus robur* 'Fastigiata' under outdoor mist in midsummer after treatment with IBA at 20,000 ppm; and long (90 cm), semi-hardwood cuttings of Q. robur 'Fastigiata Koster', treated with 5000 ppm IBA and propagated under high pressure fog systems.

Recent work on Q. *robur* in the western Himalayas (Iqbal et al 2015), showed that Indolebutyric acid (IBA) with concentration of 10,000 ppm gave the highest recorded rooting of 51.30 per cent for softwood cuttings.

2.3.2 Cuttings from seedlings and young plants (up to 3 years)

Drew and Dirr (1989) used 3 year old plants grown under 55% shade cloth and sprayed with Triforine EC (N ,N' -[1,4-piperazinedyylbis (2,2,2trichloroethylidene)] bis[forman1ide]) at a rate of 1.3 mI/l every two weeks to control powdery mildew. Terminal cuttings were collected on each date (May 15, June 18, and August 22, 1987) from firm growth with the leaves fully expanded. Ten to 15 cm (4-6 in) long cuttings were collected, one-half of all leaves removed from the lower one-half of cuttings, and the basal 2.5 cm of the stems provided a 5-sec dip in 1.0% K-IBA. Cuttings were air dried before placement in the medium. Cuttings in containers were placed under intermittent mist (5 sec/6 minutes) from 0800-1800 hours. The bench was shrouded on the sides with clear polyethylene film and the top covered with 55% Saran shade cloth. Greenhouse temperatures ranged from 18-24°C (65-70°F) at night and 30-35°C (85-95°F) during the day. Dead leaves were regularly removed to prevent disease. At the end of each 10 week rooting period, the mist was turned off, the plastic lifted, and cuttings were not watered for one week. The unrooted cuttings withered and those that rooted could be easily determined. Tug tests midway between the 10 week rooting period indicated that most cuttings rooted within 4 to 5 weeks. Cuttings were healthy throughout the 10 week period even when they had not rooted.

Struve et al. collected, cuttings on 24 June from seedlings by severing the shoot within 0.5 cm of the substrate surface. One to five cuttings per seedling were made depending on seedling height. Cutting lengths ranged from 3 to 10 cm consisting of three to seven nodes. Cutting diameters ranged from 1 to 2 mm. The basal leaves were removed and all remaining leaves were cut in half before the cuttings received 5-s basal (1 cm) dips first in 0.1 M ascorbic acid and then in 500 ppm solution of the potassium salt of IBA (United States Biochemicals, Cleveland, OH). The cutting collection procedure was modified for the next two propagation events because some stock plants did not regrow after the initial cutting collection i.e. cuttings were not taken unless there were at least six nodes on the stock plant's shoot. All cuttings had at least three nodes, leaving the stock plants' shoots with three nodes. This procedure eliminated subsequent stock plant mortality for all but one stock plant. The propagation substrate was 5015 Root Cubes, an expanded foam product. Two sheets (each 3.7 cm high) were placed in plastic flats [8 · 35 · 54 cm (ht · length · width)]. Cuttings were placed one per cell. The flats were placed in a clear plastic covered propagation tent within the greenhouse. The mist was set to run 10 s every 10 min from dawn to dusk, which maintained near 100% relative humidity. Ambient minimum and maximum temperatures inside the propagation tent were 23 ± 2 _C night and 32 ± 2 _C day with a 48% reduction in light.

Struve et al. also collected cuttings at two additional times, 17 July and 3 Aug., from stock plants with one or more shoots at least 5 cm long with six or more nodes. *Q. petraea* sources had sufficient regrowth to provide cuttings for both June and August propagation dates. Only 29 of the 45 seedlings of the one of the *Q. petraea* sources had regrown sufficiently to provide cuttings at three propagation dates. For two sources, stock plant mortality was high, 43% and 18% for Q. cerris and Q *petraea*, respectively. Sufficient resprouting occurred from both Q. *petraea* and Q. pubescens sources to allow two propagation dates between June and August. The number of cuttings per stock plant was higher in June than in August for both Q. *petraea* sources.

Mishra et al (2003) Reported that cuttings of Ban Oak (*Quercus leucotrichophora* were most successful when treated with chemical formulation of 0.8% IBA+0.2% p-HBA+5% Sucrose+5% Captan.

2.3.3 Cutting material from coppice shoots

Harmer and Baker (1991) and Harmer (2010) established cuttings from coppice shoot grown from 10 year old oak trees stumped back the year previously. In order to produce as many plants as possible shoots were cut into single node cuttings by severing the stem immediately above each bud. Leaves were then cut to 30-50% of their length, soaked in a fungicide and inserted into a 1:1:1 peat:sand:perlite mixture and placed under mist in a glasshouse. The cuttings rooted within 6-8 weeks and were overwintered in a cold glasshouse, with some winter losses. The following spring they were potted into 10cm plastic pots with peat and grit and a slow release fertiliser.

2.3.4 Cutting material from serial grafting

Zas Arregui (2008) describes a serial grafting technique for mass micropropagation of *Quercus robur* 'Fastigiata' by which 170 000 grafted plants could be produced annually from 10 initial grafts. This involves grafting adult scion material multiple times from one root stock to the next until the scion is rejuvenated and suitable as cutting material. It is described as “a method of last resort”!

2.3.5 Effect of treatments to stock plant on rooting potential

Struve et al (2010) reported that when seedlings are used, stock plant environment markedly affected rooting of harvested leafy cuttings. Irradiance, photoperiod and their interactions with nutrients had marked effects on the rooting potential of leafy cuttings. A long photoperiod (continuous light) improved rooting of *Q. petraea* cuttings. Cuttings from seedlings grown under continuous light rooted in significantly higher percentages (92%) than those from seedlings grown under natural daylength (76%).

Eshed (1996) reported that gibberellin (GA3) applied as bark treatment on leafless pruned stems of 3-year-old *Quercus ithaburensis* stock plants improved (relatively poor) rooting percentage 6- to 7-fold. GA3 application also enhanced bud release and particularly shoot growth, thus increasing the number of cuttings per stock plant.

Amissah and Bassuk 2010 showed that cuttings of *Q. bicolor* rooted better (though only 46% rooting) the lower the cutting height of stock plants; and that etiolation of shoots improved rooting.

2.3.6 Effects of cutting collection time

Spethmann (1985) suggests that success with *Q. petraea* cuttings was best when cuttings were taken in early June.

Struve et al (2010) reported that the propagation window appears to be wide for *Q petraea*; cuttings from all four sources could be rooted between June and August (see table 4 above). Averaged over all sources, percent rooting was higher in August than June (97% versus 82%, respectively), but it did not differ within a source between propagation dates except for cuttings of one of the two the *Q. petraea* sources, in which it was higher in August. Stock plants from one of the two *Q. petraea* sources were vigorous enough to provide cuttings from three propagation dates: 22 June, 13 July, and 3 Aug. Stem cuttings rooted at 72%, 100%, and 83% for the June, July, and August propagation dates, respectively. Rooting was higher in July than in June or August ($P = 0.01$). The relatively late propagation date (August) did not seem to be a factor affecting rooting or cutting survival for these

sources. Few cuttings propagated in August broke bud between sticking and December, but all rooted cuttings were healthy in December.

2.3.7 Rooting medium

Spethmann (1985, 1986) reported that of the various combinations of growth hormones, growth retardants and fungicides investigated whilst propagating 26,000 cuttings of Q. *robur* and Q. *petraea*, the optimum combination was 0.5% IBA plus 10% 'Euparen' [tolylfluanid]. 1.1 peat/sand mixes worked well as well as 'Grodan' block substrate.

Spethmann and Harms (1993) reported that different concentrations of Osmocote in gravel did not influence rooting; whereas Osmocote in peat:sand increased rooting especially at concentrations of 0.5 - 2.5 g/l. Nitrophoska had a similar effect. In gravel, only a slight increase was observed, in peat:sand from 1.0-3.0 g/l had a the stimulating effect. For successful of mass propagation of oak they concluded that the addition of low levels of Osmocote 1.5-2.5 g/l is useful and that pH should be > 5.5.

2.4 Shoot growth after rooting of cuttings

Chalupa (1995) reports that for successful vegetative propagation of oak, it is important not only to achieve rooting of cuttings, but to produce plants with low mortality and rapid growth. Rooted cuttings of *Q. robur* that formed new shoots (with active metabolic exchange between root system and stem), exhibited high survival rates. Vigorous plants were produced from cuttings which rooted quickly and were capable of rapid shoot growth immediately after rooting. Shoot growth of rooted cuttings was also stimulated by mineral nutrition. Regular watering (every 2nd day) of rooted cuttings with diluted WPM (1/10 strength of macroelements), or incorporation of slow-release fertilizers into rooting mixture, enhanced root quality and stimulated shoot growth. Supplemental nutrition with diluted WPM had a favourable influence on shoot elongation. The formation of new shoots was also stimulated by supplemental lighting. Cuttings grown under continuous light (cool white fluorescent lamps) formed new shoots at higher frequency (87%) than cuttings grown under a natural photoperiod.

2.5 Transfer to nursery beds and the forest

Chalupa (1995) reported that rooted cuttings, which formed new shoots and reached a total height of 30-50 cm in the autumn, were overwintered in the rooting mixture in the same place in an unheated greenhouse and suffered only small losses. The following spring, rooted cuttings were lifted (in early June) and transplanted in the nursery, where the growth continued. Their survival rate was high (78-94%) and vigorous plants were produced during the growing season.

Spethmann (1986) showed that when rooted cuttings forced in the greenhouse are transplanted (lined out) in early spring, they are susceptible to late frost damage; it is better to transplant after mid-May under good shelter, with watering if necessary

The initial method developed by Spethmann and Harms (1993) resulted in rooted cuttings with a mean height of 15-20 cm. If too small, cuttings are often lost during nursery transplanting and weeding. After 2 additional growth periods in the nursery, which are necessary to produce plants large enough for afforestation, *Q. petraea* had a height of 50-60 cm, and *Q. robur* of 40-50 cm.

Spethmann and Harms (1993) also explored approaches to reducing production time by planting out the tallest rooted cuttings, 40—50 cm long, produced after one year, directly into the forest. Some hundred selected *Q. petraea* cuttings with mean height of 51 cm (gravel) and 42 cm (peat:sand) were directly planted in the forest, together with cuttings after 2 years' cultivation in the nursery, mean height 65 cm (gravel), 50 cm (peat:sand). In autumn 1990, survival was evaluated (Müller, 1991). Only 1/3 of the older (but smaller) cuttings survived. In contrast the taller one-year cuttings had survival rates of 73% (grown in gravel); 94% (peat:sand).

2.6 Performance compared with seedlings

Müller (1991) compared cuttings with seedlings. *Q petraea* cuttings (0 + 1 + 2) were planted in the spring of 1987, in comparison to seedlings (2 + 2), and were measured at the end of 1990. Mean height of cuttings rooted in peat:sand was 127 cm; that of the cuttings rooted in gravel was 122 cm. The seedlings 1-year-older than the cuttings had a mean height of only 109 cm. Seedlings and cuttings were from the same provenance. Evaluation of the plant habit in 1990 showed the same differences as height. Cuttings from peat:sand had 80%, gravel 69% and seedlings 55% normally formed plants (Müller, 1991).

Muller (1996) reported on the performance of 2+0 seedlings and 0+1+2 cuttings of *Quercus robur* and *Q. petraea* in Germany. The cuttings had been initially grown in the greenhouse on gravel or on peat/sand substrates. In both species the cuttings were clearly superior in growth to the seedlings. Cuttings of *Q. petraea* grown in gravel did better than those in the peat/sand substrate. The cuttings had better habit than the seedlings, many of which were forking and bushy, often with the terminal bud missing.

Harmer (2010) reported that field performance of clonal oaks were satisfactory after 17 years.

2.7 Commercial application

Chalupa 1993 suggests that a process that uses both micropropagation (to obtain stock plants from desirable individuals) and then stem-cutting techniques (for bulking up) may to have potential. Spethmann and Harms (1993) showed that oak cutting could be produced either after 2 years in the nursery; or after 1 year using a bulk propagation method involving a fog system (at the Lower Saxony Forest Research Institute in Escherode). This makes it possible to react very quickly to missing or low crops of acorns by increasing oak cuttings production (Spethmann, 1986). The following 4 parameters were found to be critical.

- Seedling age of 6-7 years limits successful and profitable propagation; but by cutting back the mother plants every March to 5-20 cm juvenility could be maintained for more years, and a good rooting percentage was obtained. 9-year-old (or more) cut-back mother plants can be used without decreased rooting.
- Inserting time is limited to 3 weeks in June, dependent upon the physiological stage of the new growth. The best time is when the leaves were still light green but already fully developed.
- Only rooting under the continuously high humidity of a fog system secures replicable success.
- Successfully overwintering up to 95% of the rooted cuttings is only possible when retaining the rooted cuttings in the rooting bed in an unheated greenhouse.

Calculation of all costs (of the one-year method) results in a price of 0.50 DM/rooted cutting which is less than that for a 1-year-old seedling.

Drew and Dirr (1989) quote cost calculations by Bassuk suggesting that an etiolated cutting can be produced for $0.49 in the field (1989 prices) and $0.55 in the greenhouse, assuming a 15% loss (this is for recalcitrant tree species in general, not oak) and that this is cheaper than grafting.

Struve et al. (2010) state that to be commercially successful, an oak propagation system would have both high rooting and overwintering percentages and include quality factors such as root distribution about the base of the cutting. Thus, the commercial yield (the percentage of cuttings with a uniform root system and high overwinter survival after propagation) is typically lower than the percentage of cuttings that rooted.

Cuttings are potentially cheaper than micropropagated plants; but costs can nevertheless be quite substantial due the costs of maintaining stock hedges, pre-treatments use of misting systems and transplanting costs.

2.8 Contact with practitioners

Two key practitioners were contacted: Prof. Wolfgang Spethman of University of Hanover (retired) and Prof Daniel Struve Prof Daniel Struve of Ohio State University.

Wolfgang Spethmann stated that the oak improvement programme in Germany followed a similar route to that currently being progressed by FTT i.e. plus tree selection, seed orchards followed by development vegetative propagation by cuttings method. The seed orchards produced acorns only after an extended period and then only in very small amounts. The acorns were used to produce stock plants for cutting propagation until they were 6-7 years old. Then they were strongly cut back to 10 cm and used for a further 4-5 years. Also 3 year old cuttings were used as stock plants to multiply them up. In bad acorn years seedlings in nursery beds were multiplied up by cuttings. During the 1980s and 1990s oak cuttings were propagated in 5-8 state forest nurseries with each station producing 20.000 to 40.000 plants per year. In recent years propagation by cutting has largely ceased because of a policy change favouring use of seed from seed stands.

Spethmann states that oak cuttings are easy to root. The main parameters for success are using a setting date in the middle to end of June when the leaves are still light green; and stock plants age (seedlings) limited to 7 years (before cutting back again). Use of a high pressure fog system that maintains humidity (> 90 % all the time until September) is important, with no ventilation of the greenhouse (it should be closed all the time). Temperature reached up to 50°C in the closed greenhouse. Overwintering was in an unheated greenhouse. Cuttings were transplanted in the field in early spring before flushing and 3 years old cuttings were used for afforestation. The price of conventional cuttings was nearly 3 times that of seedlings. Long cuttings (best circa 50 cm) were also grown, mainly for street trees.

Daniel Struve confirmed that his more limited experience of working with oak cuttings suggested that producing plants via cuttings is relatively straightforward and has considerable potential. He achieved good rooting and establishment using fairly low tech equipment in Italy.

2.9 Conclusions - cuttings

Cutting techniques have been used commercially to multiply up small numbers of valuable oak cultivars for sale; and there a two reports of cuttings production at larger scale (tens of thousands of plants) in forestry in Germany during the 1980-90s. This work was discontinued when use of

improved material was discouraged by German forest policy in the 1990s. Cuttings appear to be a potentially viable technique for plant production in large numbers, and experience from Germany suggest that this might be feasible on a commercial or semi-commercial basis.

The potential of cuttings as propagation method is hard to evaluate accurately from a literature search because of somewhat contradictory messages over the decades. Researchers involved primarily in tissue culture appear to dismiss cuttings as an approach; whereas practitioners involved in trialling cuttings generally report success.

Studies are split between forest research practitioners interested in plant supply and/or tree breeding, and horticulturalists focussed on propagating valuable varieties or rare species (and typically working on *Q. robur*). The literature gives the impression that forestry and horticultural researchers tend to work independently of each other i.e. they don't reference each other's work consistently.

Propagation using best practice for softwood cuttings from young plants or stock hedges typically gives rooting percentages between 35% and 80%; though it should be noted that these results are mainly from carefully conducted research trials, and the higher success rates (>60%) might not necessarily be achievable in commercial practice. The rooting percentage in the mass trials in Germany were 59%; and these plants typically reached lengths of 30-50 cm by the autumn and overwintered with only small losses. They were then transplanted to a nursery with 78-94% survival, and went on to produce vigorous plants.

1-3-year-old trees can be made to root at high frequencies and produced well-developed root systems. The use of cuttings from very young plants is limited however, because the quantity of cutting material which is produced by young ortet is low; though this can be overcome to some extent by hedging. Trails with hedged plants 3-9 years old (of Q *robur*) showed high success i.e. 81% of cuttings rooted. Successful cutting propagation requires rooting under the continuously high humidity of a fog system.

Long term performance of plants from cuttings appears to be at least as good as use of seedlings.

Propagating proven physiological mature genotypes is not feasible using cuttings taken directly from mature trees. Instead the best options would appear to be to:

- use seedlings grown from acorns produced by plus trees, arresting onset of physiological maturity by hedging, then conducting long-term validation trials of the mother trees (Struve et al. 2010).
- grow a small number of plants from forced epicormics shoots from branch sections of plus trees, or known good genotypes, say by micro-propagation, then multiply these via stock hedges and cuttings.

Many authors supply estimates of rooting percentage of cuttings (the number of cuttings that produce roots), but fewer provide data on the quality of the roots, or whether the roots are adequate to produce a viable plant. Even fewer provide data on plant survival and subsequent growth. There is very little data on costs of production.

3. Micropropagation

3.1 Introduction

Early efforts on micropropagation of oak focused on regeneration from callus cultures and were not successful. This was followed in the 1980s by development of a system based on in vitro multiplication of shoots from axillary buds (Chalupa 1993). Systems based on this approach have been developed for *Q. petraea* (e.g. Chalupa 1993), *Q. robur* (e.g. Puddephat et al. 1999, Vidal et al 2003), Cork Oak (e.g. Pinto et al 2011), Himalayan oaks (Purohit et al. 2002; Tampta et al. 2008), endangered oak species such as *Q. euboica* (e.g. Kartsonas and Papafotiou 2007) and valuable horticultural cultivars. This work continued with *Q. petraea* and *Q. robur* until about 2000, after which the research focus for these two species largely switched to somatic embryogenesis; though a little research on "conventional micropropagation" continues (e.g. Ostrolucká et al 2007, Gatti et al. 2015).

Standardized reproducible procedures have been developed for micropropagating *Q. robur* and *Q. petraea* by axillary shoot growth (Vieitez et al 2011). The easiest way of developing cultures is using embryos from acorns (=zygotic embryos)[2] and seedlings as explants. However progress has also been made in using tissues from mature trees, which is important because this potentially allows trees of known good phenotype or genotype to be propagated (Vieitez et al. 1994, 2009; Ballester et al. 2009).

This report only covers the details of techniques (e.g. types of culture media) in overview. Because some of the recent progress has been made on *Q. suber*, coverage of this species is included in this part of the report.

3.2 Plant material for explants

For initiation of shoot cultures in *Q. robur* and *Q. petraea* the following types of explants have been used:

- Embryos / acorns
- Shoots of seedlings 3-6-months-old
- Shoots from hedged trees or stump sprouts
- Forced epicormics shoots from branch sections
- Forced epicormics or stump sprouts from trunks induced by partial girdling of the basal part of the stem.

Martinez et al (2012) also showed that somatic embryos derived from mature trees could be used as sources for shoot cultures.

The first successes with micropropagating oak species were carried out with zygotic embryos or seedlings as a source of explants for culture initiation. Note that most of the work has been done with *Q. robur* (e.g. see table 1). Early studies on the micropropagation of *Q. robur* and *Q. petraea* used embryonic axes, shoot apices and nodal segments isolated from 3- to 6-month-old seedlings (Vieitez et al.1985; San-Jose´ et al. 1990; Chalupa 1993). Use of zygotic embryos and seedlings

[2] "Zygotic embryos" are taken from acorns; "somatic embryos" are derived from a variety of tissues via somatic embryogenesis

remain the easiest means of establishing cultures (Chalupa 2000, Vieitez 2011, Gatti et al. 2015); but recent work has also demonstrated the feasibility of using material from mature trees (Vieitez et al. 1994, 2009; Ballester et al. 2009).

Note that recent work on *somatic embryogenesis* of oak showed a decline in embryonic potential even within different stages of embryo development, with the best results from the youngest embryos (Timofte and Timofte 2010).

Explants from mature trees tend to have lower regeneration potential. When cloning mature trees the most responsive cells are tissues:

- at the root-shoot junction,
- in root or stump sprouts, e.g. coppice shoots
- burr growths on trunks and branches (sphaeroblasts)
- epicormic shoots.

In vitro plants of *Q. robur* and *Q. petraea* have been regenerated using stump sprouts and epicormic shoots from mature trees, following established protocols (Chalupa 2000, Vieitez 2011). In contrast, it is difficult to establish in vitro cultures from buds of the current season's growth in the crown (Sánchez 1991). Chalupa 1995 reports that propagation from basal shoots of adult trees was more difficult than propagation from seedlings. For example Chalupa reports:

- mean multiplication rate of cultures of adult origin was lower (by about 28%) than the rate of juvenile cultures, however, two genotypes exhibited the same proliferation rate as cultures of seedling origin.
- during rooting 68-92% of microshoots of juvenile origin (depending upon the clone) produced roots within 2-3 weeks; whereas rooting percentages of microshoots initiated from adult trees were lower by 24-78%, depending upon the clone.

Various rejuvenation or reinvigoration procedures can be applied to mature trees in order to obtain "reactive" juvenile material potentially suitable as explant material for micropropagation (Ballester et al. 1990) i.e. :

- hedging and stool bed methods, which make it possible to use preformed dormant buds that remain quiescent after early initiation. Outgrowth of these dormant buds often leads to the development of physiologically juvenile shoots, not only those located in the lower part of the trunk but also those arising higher up in the tree.
- sectioning the branches or trunk of a mature tree to induce epicormic shoots growth. The best approach consists of sectioning thick crown branches (3-5 cm) to induce the flushing of epicormic shoots (Vieitez et al. 1994, 2009; Ballester et al. 2009).

The recommended approach when using material from adult field trees as explant sources, is to use greenhouse /growth chamber forced sprouts from cut branches, instead of directly collected field material (Santos 2012). Briefly, crown branches collected between December and March, cut into 25-30 cm segments, are placed on moist perlite beds in a growth chamber, in order to induce new shoots. The flushed shoots, which develop 10-15 days after severance, are used as source of explants, which develop into shoots exhibiting vigorous growth, long internodes and leaves resembling a more juvenile looking (less lobed) type. After excision of these shoots from the original explants, they can be cultured using a horizontal culture method (described below), and give multiplication rates recorded similar to those of cultures of juvenile origin. Following this procedure,

genotypes of oak trees older than 300 years have been successfully established in vitro (Vieitez et al. 1994).

Recent work suggest that success rates with explants from mature trees can be very similar to those from seedlings. See table 1 for an overview of studies of micropropagation of *Q. petraea* and *Q. robur* up to the year 2010 (source Vieitez et al 2011)**.**

3.3 Procedures and culture media

Santos (2012) (writing about Cork oak) summarises the procedure as follows "after disinfection with sodium hypochloride, explants (with 1-2 apical and/or lateral buds) are inoculated on WPM ("Woody Plant Medium", Lloyd and McCown, 1980) medium containing benzylaminopurine (BAP 0.5 mg/L) and naphthalene acetic acid (NAA 0.1 mg/L). After multiplication and elongation, shoots are exposed to an indol-butiric acid shock for rooting. Plants in this stage are then ready for acclimatization".

Chalupa (1993) describes the following methodology for culture initiation: after removing all leaves, the axis is cut into shoot-tip and nodal segments 10-20 mm long, which are surface sterilized in 0.1% mercuric chloride solution for 20-40 min. After 3 successive rinses in sterile distilled water, the initial explants are placed on agar nutrient medium. Explants (nodal segments) are cultured in 100 ml flasks containing 20 ml of nutrient medium.

Vieitez et al. 2011 summarise the effectiveness of various media as follows: "*although the composition of the mineral media was not decisive in initiating cultures, woody plant medium (WPM) (Lloyd and McCown 1980) and Gresshoff and Doy (1972) medium (GD) were superior to other mineral media for shoot multiplication cultures". The most widely used plant regulator at the multiplication stage is 6-benzylaminopurine (BA) (used at different concentrations)".* The same media now appear to be recommended for all types of explant. Chalupa (1995) give details of the effects of different types of media on multiplication rates and the growth, morphology and rooting of the developing plantlets.

Elongation of shoots may be required prior to rooting. Elongation medium differs from multiplication medium only in that the concentration of BA is either reduced by a factor of ten or replaced by zeatin (San-José et al. 1988). The addition of zeatin to the multiplication medium improved the proliferation of American oak shoot cultures (Vieitez et al. 2009).

Table 1 Studies on micropropagation through axillary shoot proliferation in oak species of economic relevance

Species	Explant source/origin	Medium	Growth response	References
Q. robur				
	Embryonic axes and shoot tips and nodal explants from seedlings and stump sprouts	GD, BA (4.44–0.44)[a] Dipping IBA (1 g/L) 2 min	Shoot proliferation Rooting	Vieitez et al. (1985)
	Shoot tips from seedlings and grafts from mature trees	1/2MS, BA (0.44) IBA (4.9) 8 days	Shoot proliferation Rooting	Favre and Juncker (1987); Juncker and Favre (1989)
	Shoot cultures from seedlings and stump sprouts	WPM, BA (2.66–0.88) or TDZ (0.0045–0.0090)	Shoot proliferation Rooting, acclimatization	Chalupa (1988)
	Shoot cultures from seedlings and epicormic shoots from mature trees	GD, BA (0.88) GD, BA (0.08) or Z (0.46)	Shoot proliferation; recycling horizontal shoots Shoot elongation, rooting	San–José et el. (1988)
	Shoot tips, nodal segments from seedlings and stump sprouts	WPM, BA (2.66–0.88) WPM, IBA (0.98–4.90)	Shoot proliferation Rooting, acclimatization	Chalupa (1993)
	Shoot tips from forced epicormic shoots from mature trees	WPM, BA (4.4–2.2), AC (0.5%) IBA (4.6)	Shoot proliferation Rooting	Evers et al. (1993)
	Zygotic embryos	Commercial rooting powder	Ex vitro rooting	Meier–Dinkel et al. (1993)
	Shoot tips and nodal segments from forced epicormic shoots from mature trees	GD, BA (0.88) IBA (14.8) 7 days	Shoot proliferation; recycling horizontal shoots Rooting	Vieitez et al. (1994)
	Shoot cultures from forced epicormic shoots from mature trees	1/3GD, IBA (122.5) 24 h, AC (1%)	Rooting	Sánchez et al. (1996)
	Shoot cultures from seedlings	1/2GD, IBA (4.90) 7 days	Rooting	Puddephat et al. (1999)
Q. petraea				
	Shoot tips and nodal segments from seedlings and stump sprouts	GD BA (0.88) 1/2GD, dipping IBA (0.5 g/L) 6 min	Shoot proliferation Rooting	San–José et al. (1990)
	Shoot tips and nodal segments from seedlings and stump sprouts	WPM, BA (2.66–0.88) WPM, IBA (0.98–4.90)	Shoot proliferation Rooting	Chalupa (1993)

3.3 1 Problems with maintenance of cultures

The following problems are widely reported:

- culture browning and bacterial contamination due to inadequate disinfection and/or the phytosanitary conditions of the mother plant.
- toxic mainly phenolic compounds released into the growth medium leading to loss of plant material lost.

Veietez et al 2009 overcame bacterial contamination problems in their work on American oaks by a) growing the original (adult) explant material in a growth cabinet, rather than collecting in the wild. They then overcame the problem of phenolic compounds by transferring explants to a new area of the culture medium on the opposite side of its culture tube 1 day after the initiation of culture; and then transferring the explants to fresh medium every 2 weeks. The addition of activated carbon to culture medium also seemed to prevent shoots browning (Gatti et al. 2015).

Vieitez et al (2011) report that a decline in the proliferation capacity of vertically placed oak cultures after several subcultures is a common bottleneck. According to McCown (2000), the decreased capacity for proliferation may be as a result of the episodic growth exhibited by *Quercus*,

as the growth is not continuous. Ballester et al. (2009) overcame this by placing shoots of pedunculate oak (shoot explants from which the 2 mm apices have been removed) horizontally on the medium and transferring these onto fresh medium after 2 weeks, during the 4-week multiplication cycle. After this period, all new shoots longer than 10 mm were harvested and used for multiplication or rooting, and the donor shoots were recultured (transferred to fresh medium) to produce a new crop. The procedure was repeated 3 or 4 times in all and healthy cultures were maintained. It appears that placing shoot material horizontally induces stresses to which the plant responds by attempting to grow more vigorously.

Veietez et al 2009 successfully cultured shoots of 3 species of American oaks by placing them in the "stressful" horizontal position on cytokinin-containing medium with a sequence of transfers within a 6-week subculture cycle, in order to overcome the episodic character of shoot growth. During each subculture cycle, the horizontally placed explants were cultured on media containing 0.2 mg l-1 benzyladenine (BA) for 2 weeks with two successive transfers (2 weeks each) to fresh medium with 0.1 mg l-1BA, giving a 6-week subculture cycle. The general appearance and vigour of *Q. alba* and *Q. bicolor* shoot cultures were improved by the inclusion of both 0.1 mg l-1 BA and 0.5 mg l-1 zeatin in the medium used for the second transfer within the 6-week subculture cycle. Addition of AgNO3 (3 mg l-1) to the shoot proliferation medium of *Q. rubra* had a significant positive effect on shoot development pattern by reducing deleterious symptoms, including shoot tip necrosis and early senescence of leaves.

3.3.2 Rooting

Rooting of microshoots can be achieved either in vitro, or ex vitro under nonsterile conditions by direct rooting in potting mixture. Rooting of plantlets has often been a bottleneck; for example successful rooting in cork oak was not achieved until 1990 (Pinto et al 2011).

Vieitez (2011) summarises the procedure for rooting in vitro. Isolated shoots (1.5–3 cm) are placed in media containing indole-butyric acid (IBA) or naphthalene-acetic acid (NAA) for a period of 7 or 8 days, with later transfer to an auxin-free medium (Chalupa 1993; Juncker and Favre 1989; Vieitez et al. 1994; Puddephat et al. 1999). Dipping the basal ends of the shoots for 1–2 min in highly concentrated solutions of IBA (1 g/L) and transfer to an auxin-free medium was also carried out (San-Jose´ et al. 1988). However, after many experiments, it was found that the best rooting efficiency was achieved by culturing the shoots in media containing 122.5 lM IBA for 24 h, with subsequent transfer to auxin-free media containing 1% activated charcoal. For all genotypes tested, the charcoal benefited both shoot quality and root system development, and the latter was enhanced by the formation of many lateral roots (Sanchez et al. 1996; Vieitez et al. 2009). Puddephat et al (1999) describe protocols that gave 80% rooting of *Q. robur* microshoots after 35 days.

Ex vitro rooting experiments were also performed with pedunculate oak microcuttings, which were treated with commercial rooting powder, inserted in peat-perlite substrate and placed under plastic tunnels in a greenhouse (Meier–Dinkel et al. 1993). Chalupa (1995) reports that high rooting percentages of juvenile microshoots were obtained by ex vitro rooting. After auxin treatment (a quick dip of the microshoot base into liquid IBA, 1.0 g-,·1l for 1 min), microshoots were inserted into potting mixture (peat and perlite, 1:1, v/v) and kept under a plastic sheet in a humid atmosphere. Mean rooting percentages of juvenile microshoots ranged from 54 to 80% (depending upon the clone). Ex vitro rooting was less laborious than in vitro rooting.

Microshoot quality was very important in ex vitro rooting (Chalupa 1995). Small microshoots (10-15 mm long) exhibited higher mortality rates. Fully developed leaves on microshoots were beneficial to

rooting. Stem elongation and formation of new leaves stimulated adventitious root formation. The treatment of microshoots with rooting hormone was useful for increasing the speed and uniformity of rooting and the number of adventitious roots. For ex vitro rooting, humidity control was important.

Pinto et al (2011) working with cork oak showed that continuous exposure of low concentrations of IBA (Indole-3-butyric acid) or the dipping of the base of the shoots in a concentrated solution of IBA gave the best rooting results (Manzanera and Pardos, 1990; El Kbiach *et al.*, 2004; respectively).

Other factors, such as carbon source, also affect the rates of cork oak shoot proliferation and in vitro rooting (Romano *et al.,* 1995). While sucrose (3%) allowed the best shoot elongation rates, enabling an effectively higher number of shoots during media transfer, 4% glucose was the best carbon source during rooting phases. Romano and Loução (2003) also highlighted the importance of darkness during the first week of rooting which resulted in a remarkable enhancement of the rooting percentage, number of roots developed per shoot and length of the longest root.

3.3.3 Development of plantlets and acclimatisation

Shortly after adventitious root formation, active shoot growth resumes and the size of the plantlets increases substantially (Chalupa 1995). The newly formed leaves are much less susceptible to desiccation. Plantlets were grown under high humidity for 5-8 weeks, then humidity was gradually reduced to normal levels. Plantlets grown under continuous light maintained shoot growth after root formation and exhibited higher survival rates. After plantlets formed new adapted leaves on elongated shoots and reached the height of 10-20 cm, they were transferred outdoors and grown in partial shade for 2-3 months. Most rooted plantlets of juvenile origin survived (76-94%) and continued to grow.

Pinto et al. (2011) working on cork oak report that information on the acclimatization phase is scarce. Romano and Loução (2003) reported the acclimatization of cork oak well-rooted plantlets in a mixture of peat and vermiculite using incubation in a growth chamber with high humidity. Hardening of plantlets under high humidity during four weeks was found to be essential for successful acclimatization. After two months, plants were transferred to the glasshouse and the percentage of surviving plantlets after six months ranged from 60% to 72%. In a similar strategy, El-Kbiash *et al.,* (2004) reported that 92% of rooted plants were successfully potted in horticultural substrate and incubated in a chamber with a high relative humidity.

3.4 Field performance of micropropagated plants

Field growth of micropropagated oak trees of juvenile origin is comparable to that of control seedlings (Chalupa 1995). He reports that after hardening off, the plants were planted in the field, usually in early summer. Planted trees attained a height of 20-30 cm at the end of the second growing season. In the following years, there was no significant difference in growth between the micropropagated plants and control seedlings. At the end of the 8th growing season, the micropropagated trees were more than 230-290 cm high and exhibited normal growth and appearance.

Gatti and Sgarbi (2015) noted no differences in survival between Q. robur plants of acorn, seedling and mature (stem cuttings) origin.

3.5 Effects of Genotype

Shoot productivity from cultures from individual trees varies enormously – i.e. the effect of genotype. For example, Meier-Dinkel et al. (1993) established in vitro cultures from germinated acorns harvested from 11 grafted selected pedunculate oak trees growing in two stands. After 8 months, shoot productivity varied between ten and more than 1000 shoots per genotype. Rooting and survival are also strongly dependent on the genotype; for example Meier-Dinkel et al. (1993) recorded values of rooting and survival ranging between 10% and 80% for individual genotypes.

Juncker and Favre (1989) confirmed that the between-genotype differences in oak micropropagation are large enough to explain the lack of repeatability in culture establishment, subculture and rooting; i.e. that genotype differences mean that techniques that work with one batch of material may not work, or work far worse, for another.

No significant differences are observed between-provenance, while the within-provenance differences are high (Vieitez et al 2011).

However it should be noted that large genotypic effects are also displayed in acorn production, with individual mother trees contributing strongly to seed production and others barely fruiting at all, even when observed over multiple seasons. Genotye effects are frustrating because they lead to imbalances in production from a population of mother trees, which can be serious if the number of mother trees are limited. However they do not constitute a reason for dismissing vegetative propagation, it just means that programmes need to be designed with this effect in mind.

3.6 Commercialisation potential

There is strikingly little information published on costs and commercial potential of producing oak by micropropagation. Vieitez et al 2011 state that whilst micropropagation is often effective, even with adult material, techniques are not sufficiently cost-effective to be applied on a commercial scale[3].

Several technical imitations to application of the technology at a commercial scale have been identified i.e.: 1) the effect of genotype, 2) a progressive decline of shoot proliferation during successive subcultures and 3) difficulties in propagating mature selected trees lacking stump sprouts or epicormic shoots (Vieitez 2011). Other authors have stated that variable success in rooting remains an issue. It also appears that the sheer amount laboratory time involved results in high costs per seedling.

Trevor Fenning (Forest Research) estimated the likely approximate costs of micro-propagated plants at Forest Research might be £10-£20 each (see appendix 2). Specific oak cultivars are offered for sale on the web at high unit process (£10-70 per plant dependent on size), though it is not always clear if these have been vegetatively produced.

Chalupa (1995) states that using "*micropropagation in combination with stem-cutting techniques will perhaps enable the development of an integrated system to be used for mass propagation of*

[3] Note that in cork oak, for which commercial production of selected clones is most advanced, only few reports were based on conventional micropropagation, and research has been strongly focused on SE (Pinto et al. 2011).

selected oak clones; for example, micropropagation may provide the initial multiplication stage prior to stem cutting propagation".

3.7 Protocols for other oak species

Recent (post 2010) work to establish protocols for other oak species, which might be worth following up for updates to techniques include:

- Holm oak (Q. ilex) - Linian et al (2011)
- *Quercus shumardii* - LuXiuLi et al (2015)
- Mexican Oaks - Delgadillo-Díaz de León et al (2013)
- Tasar Oak – Aseesh and Tamta (2014)

3.8 Contact with practitioners

The following commercial micropropagation businesses were contact: Gentech Propagation Ltd, Dundee (Nigel Ebblewaite) and Micropropagation Services, Loughborough (Barbara Wright). Gentech stated that they had no expertise with *Quercus*; but if they could be supplied with suitable initiation material, they could attempt to initiate it into sterile culture. They would also be interested in any information regarding techniques that may be employed to achieve successful micropropagation. Micropropagation Services stated that they did do some oak micropropagation 10-15 years ago but did not find it anywhere near being an economic proposition; and that their business has changed and they would not be interested in looking at this further.

Ana Vieitez of the Instituto de Investigaciones Agrobiológicas, Spain was contacted (amvieitez@iiag.csic.es) to ask if she was aware of any commercial ventures in oak micropropagation but no response was received. We were unable to find contact details for Vladamir Chalupa.

3.9 Conclusions – micro-propagation

Micropropagation systems have been developed for *Q. petraea* and *Q. robur*, but appear only to have been used for research purposes, rather than in any form of commercial production. We were only able to find one commercial company with experience of oak micropropagation in the UK, and their view was that it was not commercially viable, and they were not interested in further work. However micro-propagation could still be of potential use; for example for quickly multiplying up particularly rare acorns to produce plants for stock hedges.

There appear to be reproducible protocols, but these are still subject to considerable variation in success due to the effect of genotype (differing success rates with individual trees). Micropropagation appears to be effective, even with adult material, but techniques are not sufficiently cost-effective to be applied on a commercial scale. Note that work on "conventional micro propagation" continued with *Q. petraea* and *Q. robur* until about 2000, after which the research focus for these two species largely switched to somatic embryogenesis.

Protocols are based on in vitro multiplication of shoots from axillary buds. The easiest way of developing cultures is using embryos from acorns (=zygotic embryos) and seedlings as explants. However progress has also been made in using tissues from mature trees, which is important because this potentially allows trees of known good phenotype or genotype to be propagated. The recommended approach when using material from adult field trees as explant sources is to use

greenhouse /growth chamber forced sprouts from cut branches, instead of directly collected field material.

Problems of bacterial contamination of cultures and release of toxic mainly phenolic compounds into the growth medium have both largely been overcome; the latter by transferring explants to a new area of the culture medium 1 day after the initiation of culture; and then transferring the explants to fresh medium every 2 weeks. Declines in the proliferation capacity of oak cultures after several subcultures is a common bottleneck. It appears to be possible to overcome this by placing shoots horizontally on the medium (explants are traditionally orientated vertically) and transferring these onto fresh medium after 2 weeks.

Rooting of microshoots can be achieved either in vitro, or ex vitro under nonsterile conditions by direct rooting in potting mixture. Rooting of plantlets remains something of a bottleneck, with variable success. Ex vitro rooting was less laborious than in vitro rooting. During acclimatisation plantlets involves growing them under high humidity for several weeks, then gradually reducing humidity was to normal levels. Field growth of micropropagated oak trees is comparable to that of control seedlings.

There is strikingly little information published on costs and commercial potential of producing oak by micropropagation. Trevor Fenning (Forest Research) estimated the likely approximate costs of micro-propagated plants at Forest Research might be £10-£20 each (see appendix 2). Specific oak cultivars are offered for sale on the web at high unit process (£10-70 per plant dependent on size), though it is not always clear if these have been vegetatively produced.

4. Next steps

Cuttings appear to be the only potentially viable technique for plant production in large numbers, and experience from Germany suggest that this might be feasible on a commercial or semi-commercial basis. So the main conclusion of this review is that FTT should consider developing cuttings expertise based on the German experience.

The process for plant production could be:

1. To collect acorns from the BSOs as soon as they start fruiting and use these to produce stock hedges.
2. For clones where acorns were particularly scarce, it might be sensible to multiply these up by micropropagation. This would depend on whether if this was feasible at reasonable cost - the advantage being that acorns could be multiplied up more rapidly by micro-prop than by using cuttings techniques, and this could shorten the project time scales.
3. Carry out research with cooperation of FTT nurseries to develop cuttings expertise and nursery protocols.
4. Make the stock plants available to nurseries for propagation.

However there may also be a limited role for micro-propagation of semi-mature or mature plus trees, to produce further stock hedge plants, if this was thought to be a useful addition to any production programme.

One drawback with the use of cuttings is the space requirement for the stock hedges, and the costs of their maintenance. The costs of plants produced as cuttings, estimated to be about 3 times

seedling costs, is also an issue; and it is hard to know how the market might react to stock at this price.

This proposal to develop cutting expertise should be critically assessed by the Oak Group. A first step would be for FTT to start a dialogue with field staff who were involved with the German programme, and if this continues to be useful, to organise a trip to Germany.

Acknowledgements

The author wishes to thank Steve Lee and Trevor Fenning (Forest Research), John Fennessy, Wolfgang Spethmann (University of Hanover) and Daniel Struve (University of Ohio).

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Appendix 1 Email correspondence with key practitioners

Cuttings

Wolfgang Spethmann

I'll tell you something about my experiences with oak cuttings: end of the 1970s I developed successful mass propagation of oaks at the Lower Saxony Forest Research Station Escherode. Our head was Dr. Jochen Kleinschmit. In the following years we propagated some hundredthousands of oak cuttings. They were planted successful in our forests. A lot of investigations you can find in a booklet:

SPETHMANN, W. (1986) Stecklingsvermehrung von Stiel- und Traubeneiche (*Quercus robur* L. und *Quercus petraea* (Matt.) Liebl.). Schriften der Forstlichen Fakultät der Universität Göttingen und der Niedersächsischen Forstlichen Versuchsanstalt Band 86 , pp.99

The method has been optimized in the following years by fertilization and low pH of the rooting substrate. Since 1987 to 2010 I was a professor for woody plants research at the University of Hanover. A lot of research and PhD and diploma thesis deal with cuttings of many different woody plants. My latest success was the development of long cuttings, and with oak too (50-150cm), in roses we rooted cuttings of 350cm length (see my paper of 2007).

At first our situation in forestry 45 years ago. At that time forestry breeding started in a large scale. At first spruces. Strong selections in seedling beds of improved provenances. Cutting propagation of estimated 2 million plants a year. Seed orchards and so on. Afterwards breeding of broadleaved started in the same way, you also started now. Plus tree selection, seed orchards and development of a cutting method. Seed orchards have produced acorns very late and only in very small amounts. Not enough to use the acorns for conventional afforestation. But we use them as stockplants for our cutting propagation until they are 6-7 years old. After a strong cutting back to 10 cm these seedling plants were used for further 4-5 years. Also 3 years old cuttings could be used as stockplants to multiply them.
In bad acorn years we multiply seedling beds by cuttings too. In the 80s and 90s so oak cuttings were propagated in 5-8 state forest nurseries. In each station 20.000 to 40.000 a year. After the time of forest decline end of the 80th forest policy changed totally in the 90s. Breeding programs were reduced. Aim was to plant stable forests, mixed forests, Stop of spruce cutting propagation. In oak natural propagation in stands instead of planting takes place. And after that time cutting propagation of oaks had been shortened totally after the 90s years. I asked my forest colleague for stations with oak cutting propagation. He said today there is no station propagating large amounts. For us oak cuttings are easily to root. Three parameters are important for success: setting date middle to end of June. Leaves should be still light green; stock plants age (seedlings) up to 7 years. And most important: a high pressure fog system, humidity more than 90 % all the time until September, no ventilation of greenhouse. It should be closed all the time. Temperature could be up to 50°C if the greenhouse is closed! Overwintering in an unheated greenhouse. Cuttings were transplanted in the field in early spring before flushing. 3 years old cuttings were used for afforestation.

As I remember the price of conventional cuttings was nearly 3 times as seedlings. Long cuttings are only a little more expensive. Setting time middle to end of July. Only this years shoots could be set. Good size is 50 cm or more. Advantage of long cuttings is that they grow direct terminally, whereas conventional cuttings 2-3 years grow like small bushes. Stock plant age of long cuttings could be

more than 8 years especially when they were cut back strongly, they grow up to 2 m in 3 years! We recommend long cuttings to propagate street trees.

I hope that some of your questions could be answered. Contact in Germany regarding forest tree breeding could be the Hessische Forstliche Versuchsanstalt, D-34346 Hannoversch Münden, Prof. Oelkers Str 6, (some years ago combined with the Lower Saxony Forest Research Station).

Regarding our other projects we have experience of cutting propagation too with Prunus avium, Acer platanoides/pseudoplatanus, Betula pendula/pubescens since long time.
In the Hessische Forstliche Versuchsanstalt (correct name today: Nordwestdeutsche Forstliche Versuchsanstalt!) they have also experience with in vitro propagation of oak, Prunus avium, Betula and maple (Dr. Meyer-Dinkel is responsible).
At the Versuchsanstalt contact Person for cutting propagation is Dr. Jörg Kleinschmit (joerg.kleinschmit@nw-fva.de), for breeding projects: alwin.janssen@nw-fva.de.

Daniel Struve

Dear Dr. Worrell:

I think the potential for producing cuttings from young seedlings of Q. *robur* and *petraea* is great. The link below describes the methods used for *Q. cerris, pubescens and petraea* under low tech conditions in Florence, Italy:

http://hortsci.ashspublications.org/content/45/11/1729.short

We had high rooting percentages with two and tree flush seedlings; some of the cuttings were rooted within 10 days. We used high humidity tents with low frequency misting (4x per day). A key component to the system was Oasis Root Cubes as opposed to peat-based substrates. There has been reports of the difficulty of establishing cuttings rooted in Root Cubes in substrates after rooting, but I did not find that a problem. Also, a pretreatment with 0.1mM ascorbic acid was used. Although not fully documented, earlier research indicated that a 10 second basal dip in ascorbic acid and then a 10 second basal dip in IBA gave good rooting, rapid bud break after rooting and high overwintering survival. Also, much lower concentrations of IBA could be used with the ascorbic acid pre-dip. The hypothesis was that the ascorbic acid interacted with peroxidase and phenolic compounds (which inactivate IBA) and thus we could use lower concentrations of IBA.

Seed production can be stimulated by close spacing in seed orchards. Our work (never published) with grafted black walnut seed orchards showed that when clones were planted on close spacing (2 m within row, 3 m between rows) they began to bear fruit within two years of planting; the same clones planted on wide spacing (4 x 4 m spacing) did not begin to bear before year 5. Clones trained on trellis also began to bear two years after planting, and produced more fruit than those on close spacing but not trellised. Cumulative 10-year seed production (the length of the study) was highest in the trellised orchard. However, if conducted longer, I think the close spacing planting orchard would have produced more seed than the trellis system, due to greater size. The trellising technique trained the lateral branches into three horizontal levels, thus stimulating reproductive growth. Horizontal limb training and close planting have been used in the fruit industry to stimulate reproductive growth; we just applied those observations to the black walnuts. I think similar treatment of the oak seedlings will give similar results.

In the US *Q. robur* begins to bear fruit as easily as 3 years from germination. In Germany, they claim the robur don't begin to bear until age 50. The precocity in the US is due, in part, to unintentional selection. The first *Q. robur* plants in the US (three individuals) were planted on the Michigan State University campus in East Lansing, MI. Seeds were collected from those trees and sent to a commercial nursery to produce whips with the understanding that the first whips would be sent back to MSU. The first trees were also planted on MSU (about a mile of them on 10m spacing on oppsite sides of a road. When the MSU progeny began to bear, seeds were collected and sent to the commercial nursery. Thus, the early seed producing individuals were pollination each other, fixing the early bearing habit in the subsequent progeny. I offer this as a cautionary consideration in your operational activities. The other question about seed orchards is to determine if random pollination among the individuals within the seed orchard occurs. I'm guessing it doesn't due to phenological differences among the individuals within the orchard.

Hedging is a possibility, but it is a significant commitment with regard to space, but has real advantages in management and maintenance of ortet-ramet identity. Another possibility is to collect lateral shoots from your production blocks. With the Italian work I did, I found that lateral shoot development occurred frequently in the rooted cuttings.

I hope my comments have helped. If you have additional questions, please email again. Of course, I'd enjoy a site visit, but recognize the expense of such a visit. Happy propagating.

Dan Struve

Daniel Struve, Professor Emeritus
Department of Horticulture and Crop Science
The Ohio State University
386 E Torrence Road
Columbus, OH 43214
614-313-5975
dstruve51@gmail.com

Micropropagation

Dear Steve,
I would be very interested in discussing the possibility of Oak tissue culture. To date we have had success with a Betula nana tree that was sent to us but have no experience of any of the other genera you mention below. We did produce 140,000 Populus trees last year and annually around 800,000 soft fruit plants, all derived from Nuclear stock plants. If you have some suitable initiation material we could attempt to initiate into sterile culture or if you have any information regarding techniques that may be employed to achieve successful micropropagation of these genera I would be very interested. Please do pass on my contact details to Rick.

Nigel Ebblewhite
Manager
Gentech Propagation Ltd
Dundee
DD2 1SW
01382 562644
nigel@gentech.sol.co.uk
www.gentech.org.uk

Hello Steve
Thank you for getting in touch with us about this. We did do some oak microprop 10-15 years ago but did not find it anywhere near being an economic proposition. Unfortunately our business has changed quite a bit and we would not be interested in looking at this further.
I'm sorry that we can't be of help but I hope your project does progress.

Regards
Barbara
Barbara Wright (Mrs)
Micropropagation Services (EM) Ltd
Kirk Ley Road, East Leake, Loughborough, LE12 6PE
Tel: (01509) 856295

Appendix 2

Possibilities and Issues for using tissue culture approaches for the micro-propagation of the European oak (*Quercus robur* L.) at FR-NRS

By Trevor Fenning, FR-NRS, 5th November 2013

Introduction

The European oak (*Quercus robur* L.) is one of the UK's most iconic and important native deciduous tree species and it is highly valued for the quality of its timber as well as for its wildlife, landscape and recreational value, especially in the centre and south east of Britain where it is very abundant.

Unfortunately, *Q. robur* is also known for its long rotation period and a long juvenile period (in excess of 60 years is not unusual), as well as for its erratic and relatively modest seed set thereafter. This makes the management of seed stocks for supplying replanting schemes difficult, especially in relation to arranging a reliable supply of suitable planting material that complies with the requirements of the UK's native seed zones. This problem is made worse by the fact that *Q. robur* is also difficult to manage in a nursery setting too, with relatively poor rates of success at producing rooted cuttings being the norm apparently (Vieitez *et al*., 2012).

Tissue culture approaches offer a means for overcoming some of these problems, for instance by enabling the micro-propagation of additional plant material according to need from those seeds (acorns) that are available, as has been achieved with many other tree species. As this is a common problem for all *Quercus* ssp., repeated attempts have been made by many groups across Europe to develop suitable methods for doing this with various oak species (including *Q. robur*) over the past 30 years.

While it has certainly proved possible to micro-propagate shoots from various oak species (especially when starting from zygotic embryos), multiplication rates remain stubbornly low and the members of this species group are regarded by the tissue culture community as being amongst the most technically demanding and recalcitrant of tree species to work with.

For instance, although various approaches have been utilised to produce micro-propagated shoots from assorted *Quercus* ssp., some of which have gone on to be planted in small scale experimental field trials, to date there has not been even a single full scale trial of any kind with plant material produce by these means for any European or North American oak species (Vieitez *et al*., 2012), and I am not aware of any being planned, and there are also very few groups in Europe still working on *Quercus* ssp. at this time.

From this it is apparent that although it is certainly possible to use tissue culture approaches to alleviate the limitations to the supply of native *Q. robur* material, it is likely to be difficult and the associated costs may well also be a significant barrier. Working from what is known about the success or otherwise of such approaches for *Q. robur*, there now follows a brief analysis of the resources at probably will be needed if it were considered desirable to undertake such a programme of work at FR-NRS at some point in the near future.

Vieitez *et al*., (2012). Application of biotechnological tools to *Quercus* improvement. *European Journal of Forest Research* 131 (3), 519-539. [Review article]

Resource requirement at FR-NRS

This assumes that shoots will need to be produced from not less than 50 cell culture lines of *Q. robur* (to be established from acorns), each of which will be capable of delivering approximately 50 – 100 rooted plantlets at a time (i.e. ~2,500 – 5,000 in total), in 2-3 batches per year, *or* 4-6 batches per year (~5,000 – 10,000 plants) when the Grodome is available, as this will enable the year round production of plants.

One technician for a minimum of 3, but preferably 4 days a week, at pay band 5 or 6.

This will allow for the time needed to keep up with that amount of material, including making the media and keeping the lab organised (including paperwork and record keeping). At FR charge rates, this is likely to cost between £45k and £60k in annual salary and overhead costs for the technician (plus not less than another £15k - £20k in annual supervision costs, although based on our experience with ash so far, this may be a rather conservative estimate!). However, it might be possible to employ someone on another sort of contract possibly, or to configure this as a student project to start off with perhaps.

Lab and other associated costs in the order of £15k – £20k per year.

It is hard to be sure about this without being more familiar with the precise demands of working with this species, but the lab costs are unlikely to be less than what has been allowed for a similar scale of work in WP6 of the *Living Ash Project* and will probably be more, as that project is working with a much more amenable species. Note that this cost *does not include any nursery charges*, which are likely to increase this total.

6 - 10 m^2 of shelf space in Growth Room 6 (at 25 ^oC, 16 hours daylight, 8 hours dark).

This is based on operating the standard tissue culture arrangement of producing 6 jars of fresh shoots at each subculture and 6 jars of older shoots for producing the rooted shoots etc. per *in vitro* shoot line, which equates to 600 jars allowing for all 50 shoot lines, plus however many extra may be needed for allowing the plantlets to actually produce roots before being transferred to the nursery. Up to 100 jars can fit on 1 m^2 of shelving space in GR6 (with no space between them), and so this implies a *minimum space requirement* of between 6 and 10 m^2 of shelf space.

All these figures suggest a production cost for the plants in the order of £10 - £20 each.

And this is once we are fully familiar with the demands of working with *Q. robur* and the production systems are de-bugged - which is likely to take some time, especially for a demanding species such as this.

Other issues

It would also be desirable to purchase at least one new large laminar air flow bench (Cost ~£10k), in order to ensure that there is sufficient working area in the clean lab for this project without compromising the existing work demands. There is just about room enough for another clean bench note (or else the older of the two could be replaced, but that is useful for pouring media etc.), but it would be cosy!

There is probably sufficient space in the media prep area of lab 11 for the increased work activity that is likely to be associated with running another project such as this, without conflicting with the existing project work, but there will be a problem in the culture rooms i.e. the available space there is already fully committed to the existing programmes of work for Sitka spruce and Ash. Based on the current work plans for those projects (which includes a certain reduction in the space requirement for Sitka spruce material), it will not be possible to undertake a new project of this type without having a major impact on our ability to meet our current objectives for one or both of these other project areas.

The need to replace the existing culture rooms with 2 or 3 new ones has already been raised in a discussion document (dated 25th October 2013), and so it should be possible to address this deficiency as part of such a plan of work, but this will take some time to organise and to obtain the necessary capital funding from Defra, and then to execute the change over. I am assuming that this process will take not less than 2 years and probably nearer to 3 to complete, even if Defra agrees to provide the necessary funds relatively quickly and without imposing any delays.

In summary, while it is technically possible to use tissue culture methods to alleviate the supply problems of native *Q. robur* material, the technical challenges that need to be overcome in order to achieve this are formidable and together with the high costs associated with that, major barriers remain to developing this approach as anything more than an interesting research tool.