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# OAK PROPAGATION TECHNIQUES

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## Introduction

The propagation of *Quercus* species for forestry and horticultural end uses historically has been a challenging task. However, successful advances in the propagation of this genus have been demonstrated recently by several research institutions in the areas of grafting, softwood cutting propagation, and tissue culture technologies.

It is important to realize that there are relatively few plant propagators who specialize in *Quercus* species. As a result, successful examples of oak propagation technologies can be cited, but there remains much to accomplish in terms of the refinement of techniques to the point of commercially acceptable levels. While many challenges remain, it is a fact that the propagation of oaks by various means has been demonstrated to be biologically possible. It remains for us to build upon these successful examples and work to develop repeatable propagation techniques for the genus. These are certainly exciting times to work with oak, arguably the single most important tree genus in the world.

The purpose of this paper is to provide a short review of oak propagation techniques that are currently being employed at both the research level and in commercial nursery operations. The presentation of these descriptions should provide an insight into some of the factors required to produce high quality oak propagules by either seeds or vegetative means.

## Seed Propagation

Many people are oriented towards the propagation of *Quercus* species via seeds. This propagation method sometimes can be difficult due to the recalcitrant nature of oak seeds. High moisture content and respiration rates are required within the seeds to allow for successful germination to occur in the spring, and the seed storage requirements of the genus practically eliminate the possibility of multiple-year storage.

The successful manipulation of recalcitrant oak seeds to produce high quality seedling stock either in bare root nurseries or in container systems has been the traditional method of oak propagation worldwide. For reforestation programs in the U.S., the production levels of *Quercus* are quite low. In 1990, 13.8 million seedlings were produced, mainly *Q. rubra*, *Q. alba*, *Q. macrocarpa*, *Q. palustris*, and *Q. velutina* (Steiner, 1993). This is a very modest level of production in contrast to the 140 million loblolly pine (*Pinus taeda*) seedlings produced in the southern U.S. that same year.

Since oaks are more difficult to grow in comparison to other tree genera, a focus on seed quality has been an important part of our bare root nursery operations in Indiana. In the midwestern U.S., our primary seed pests are acorn weevils (*Conotrachelus* and *Curculio* spp.), which can have a devastating impact on seed quality, especially in poor seed years. After the adult female lays an egg within the acorn, the resulting larva will feed on the cotyledons, and many times will destroy the seed. In addition, fungal pathogens, primar-

ily *Fusarium* spp., also can be troublesome. These biological agents will have a variable impact on seed quality depending upon the species involved, location, and seed year.

Between trees within a species there can be tremendous differences in seed size and appearance. Seed differences can be dramatic even within a single tree. Large seeds normally will produce large, more vigorous seedlings in the nursery. However, this "maternal" effect does not last for the life of the tree, but is lost several years after field establishment.

Quality-oriented nurseries focus on seed quality a great deal. Sampling (via cut tests) will reveal the percentage of good seed in each seedlot. The total number of seeds is determined by weight and volume calculations and the cut test information is then used to calculate a precise sowing rate in the nursery bed. This procedure is laborious and expensive, but it does result in an exact knowledge of what is sown and what will emerge in the spring.

### **A. Bareroot Production**

Approximately 1 million oak seedlings of about 12 species are produced per year in the State of Indiana nursery system. Standard nursery practices include: soil fumigation with methyl bromide to destroy most weed seeds and fungal pathogens in the seedbeds; sowing to exact density requirements (5-7 healthy seeds per square foot); and root-pruning in early summer to produce a fibrous root system. Other nursery operations, including fertilization, pest control, and irrigation, are conducted as needed, depending upon the species involved. Seedlings are lifted in the fall season, graded for size and stored in a cooler above freezing. Shipment to our 10,000 customers occurs in the early spring.

The biggest, single factor in producing quality oak seedlings is seedbed density. High quality stock cannot be grown at higher densities by compensating with fertilizer or irrigation. Since root systems are the key to outplanting performance, low densities will allow for the development of quality root systems. These low seedbed densities are combined with a single undercutting (or root wrenching) treatment at the completion of the first growth flush. Undercutting has been shown to result in a doubling of fibrous lateral roots, and a more "balanced" seedling with an improved root-shoot ratio. The timing of the undercutting to coincide with the end of the first flush is preferable to a later date because the root systems are much easier to cut and fewer seedlings are severely damaged.

### **B. Container Systems**

Intensive culture of seedling oaks for landscape purposes is becoming quite popular in the midwestern United States. The process usually involves the sowing of pre-stratified acorns into small containers (2.5 x 6, or 2.5 x 8 inches) in a greenhouse or polytunnel, in early March. Up-canning to a 1 or 2 gallon container is done after the completion of the second flush occurs in May. Trees are also staked at this time. After the first growing season, the trees are up-canned again to a 5 gallon container to grow on for a second growing season.

The most common container medium used is a combination of pine bark, peat, and sand (3:1:1). In addition, Osmocote (18-6-12) fertilizer is used along with weekly applications of liquid (30-10-10) fertilizers.

At Ohio State University, a production system using these procedures have resulted with a potted liner 5' tall in 7 months (Struve, et al., 1987). In addition to heavy fertilization regimes, the containers are treated with a copper carbonate solution which causes a chemical "root pruning" effect. These more fibrous root systems result in significantly improved field performance.

### **Vegetative Propagation**

The propagation of *Quercus* by a number of vegetative means has been shown to be realistic for most species. It can provide for the efficient utilization of valuable germplasm in both the commercial forestry and horticultural industries. In addition, vegetative propagules are a desirable alternative to traditional seedlings, since the reliance on infrequent seed crops can be avoided.

#### **A. Grafting and Budding**

Grafting is an ancient form of plant propagation which has been shown to be successful in *Quercus*. Valuable germplasm can be replicated and preserved by grafting onto potted seedling rootstocks in a greenhouse in the early spring.

The following is a description of the grafting techniques employed for the oak species included in the Indiana Division of Forestry Tree Improvement Program. Success rates of over 90 percent have been obtained repeatedly by using this technique.

Dormant scionwood is collected in late winter and stored dry in a cooler above freezing. It is important to use scionwood obtained from the previous growing season. In addition, avoid the use of terminal buds, since scions with lateral buds will be more successful. Rootstocks are potted up either in the previous year or in late winter. Temperature regimes within the greenhouse range from 65° F (18°C) at night to 95° F (35°C) during the day. No supplemental lighting is required.

Grafting should be timed to coincide with the onset of budbreak on the rootstock, rather than full leaf expansion. Graft carpentry is fairly straightforward -- cleft, side, and whip grafts are all successful. However, the use of a modified side graft which employs a short scion (1.5 inch) with a single lateral bud placed into a corresponding side cut on the rootstock, just above the soil line, has been shown to be a very rapid and successful technique for grafting oaks. For side grafts, the top of the rootstock is removed 10 days after grafting. Depending upon the temperature regimes within the greenhouse, shoot expansion on the scion should commence in about 10-14 days.

Budding bands and Parafilm (or grafting wax) are used to bind the graft and prevent desiccation. It is desirable to completely cover the entire scion with Parafilm, including the lateral bud. Chip budding onto potted rootstocks in a greenhouse has also been used for *Q. rubra*, as an alternative to grafting, when scionwood is limited (Zaczek, 1993).

Literature indicates that dormant field budding has not been successful commercially, but this could be dependent upon the expertise of the propagator and the species involved. The cool, moist conditions found in Britain and on the West Coast of North America are suitable for field grafting onto established rootstocks. Scionwood is collected in March and cleft-grafted in the field when the understock buds begin to swell (Dirr and Hauser, 1987).

In Ontario, a novel approach to grafting *Q. robur* var. *fastigiata* by the use of root pieces was reported by Leiss (1984). Root sections (6 inch) were potted up in December in a cool greenhouse and covered with damp peat, except for the very top. New root growth was initiated in 3 weeks and grafting began in mid-February. Side veneer grafts were used. The entire root piece was covered with damp peat and placed inside a grafting case under shade. Greenhouse temperatures were 68°F. The grafting case was gradually opened after the first 2 weeks and the fully acclimated grafts were outplanted in late May. Scion growth was very uniform, with no suckering encountered. This same technique was attempted for *Q. rubra*, but was not successful.

Nurse-seed grafting also has been employed in *Quercus*. This technique utilizes a newly germinated seedling as an understock source and possibly can be considered a form of rooting. Newly emerging plumules are decapitated prior to leaf development, and the hypocotyl is then split with a grafting knife. The small diameter hypocotyl is cleft-grafted, and carefully wrapped with cotton string. Scions are collected in the spring season at the onset of budbreak. The grafts are potted in containers with the graft union well covered and placed inside a polytent under shade. As shoot development begins on the scions, the polytent is vented gradually. This technique has been successful (50%) for both *Q. montana* and *Q. palustris* (Goggans and Moore, 1967). It also has been reported as a successful grafting technique for *Castanea* and *Camellia* species in which root formation actually developed from the scions.

Regardless of the grafting method used, there can be long-term problems in the field, especially in the red oak (section *Lobatae*) (Santamour, 1988). While graft incompatibility is a definite phenomenon in the red oak group, it is not as great a problem in other oaks (Santamour, 1983).

Research conducted by Santamour (1983, 1988) has resulted in a theory which coincides that long-term graft compatibility in oak is dependent upon the specific relationship between the scion and stock for a particular enzyme. The cambial peroxidase isoenzyme has been identified as the primary agent in oak that mediates in the conversion of cinnamic acids into lignin, and the subsequent bonding of lignin to carbohydrates. Without lignification, long-term graft survival is not possible. This same enzyme is also important in *Acer* and *Castanea*.

For the red oak subgenus, 9 different phenotypes of the enzyme have been identified by use of starch gel electrophoresis analyses. Graft failure can be expected unless the scion and stock possess identical forms of cambial peroxidase enzyme. Such failure can take up to 5 years to become evident. Symptoms of incompatibility include precocious flowering, vigorous rootstock suckering, constricted diameter growth of the rootstock in comparison to the scion, and short internode lengths.

In the Indiana *Q. rubra* breeding population, 139 incompatible grafts have been typed, representing a total of 47 different clones. Analysis revealed that 82% of these grafts could have been predicted to fail based upon gel electrophoresis analysis (unpublished data). Of the 805 grafts in the Indiana collection, representing 180 different clones, 17% were judged to be incompatible in 1992 (at age 8).

For the *Quercus* section *Quercus* (formerly subgenus *Lepidobalanus*), there is a single, predominant phenotype across all species, and therefore not as many instances of graft incompatibility should be encountered. Other subgenera have not been adequately sampled to date.

## **B. Softwood Cuttings**

Successful production of rooted *Quercus* cuttings is a reality. While the genus is not “easy” to propagate from cuttings, it still is possible to obtain commercially acceptable results for some species.

Since oak is a challenge to propagate from softwood cuttings and impossible to root from dormant, hardwood cuttings, the propagator must time the collection of cutting material exactly to maximize results. Optimal timing of cutting collection is a fairly narrow window of 2-3 weeks and coincides with the completion of the first flush, but before the onset of the second flush (LAG phase), (Teclaw and Isebrands, 1987).

Maynard and Bassuk (1987) conducted a series of experiments with several difficult-to-root genera including *Quercus*, and determined that rooting success could be enhanced through the use of various light-exclusion treatments. Stock plant shoots were grown in the absence of light (etiolated), banded with small strips of Velcro treated with IBA in talc at the point where the cuttings would be struck, and then moved into the light to develop normally. These types of stock plant manipulations resulted in significantly higher rooting percentages for *Q. coccinea*, *Q. palustris*, and *Q. robur*. There were, however, no significant treatment differences detected for *Q. rubra*.

Perhaps the greatest influence on rooting is stock plant age. Seedling-origin material can root at very high percentages (90%), but desirable, mature trees are not so simple. Rooting percentages of softwood cuttings collected from mature trees are normally very low or nil. Furthermore, those cuttings that do root can maintain their mature growth characteristics such as slow growth rates, and possibly, plagiotropic growth habits. While trees older than 4-6 years will root with difficulty, the percent rooting is not only dependent on age alone, but also on the clone (Zaczek, 1993).

Serial propagation of oak, by means of repeated grafting cycles, has been reported to induce “rejuvenation” in *Q. virginiana* (Morgan, et al., 1980) and *Q. acutissima* (Moon and Yi, 1993). In addition, the use of hedging (cutting back of stock plants in late winter to early spring) has resulted in robust growth and cutting material that roots at higher percentages (Chalupa, 1993). Hedging may not induce rejuvenation of the desired clones (Zaczek, 1993), but at least will arrest the maturation process. Stump sprouts from mature, elite trees in Europe also have been utilized as cutting material (Chalupa, 1993).

Vigorous stem-cutting material is collected at the lag stage between the first and second

flush. Later flush material normally roots in lower percentages (Drew and Dirr, 1989). Cuttings are prepared in a similar fashion to other softwood cuttings: five to six inch long cuttings are made with 3-5 leaves per cutting. The leaves are trimmed in half. The cuttings then are dipped in one of several hormone preparations such as IBA in talc (1.0% + .01% NAA), 1.2% IBA + EtOH 5-second dip, or 1.0% K-IBA 5-second dip. Many different hormone treatments have been used for oaks. Cuttings are placed under a polytent in a shaded greenhouse with intermittent fog or mist. Fog has been reported to be a better source of moisture for both *Q. robur* and *Q. rubra*. Depending upon temperature, rooting can occur in 5 weeks. Greenhouse temperature regimes normally are between 18-24° C (65-70° F) night and 30-35° C (85-95° F) day. Rooting percentages are dependent upon the clone and year.

Following rooting, the stimulation of shoot growth is desired in the greenhouse. Successful overwintering of the cuttings is dependent upon having an adequate carbohydrate reserve available to insure regrowth in the spring. In general, oak cuttings that root rapidly (4-5 weeks), and early in the growing season, will be the most likely to develop new shoot growth and be successfully overwintered. Treatments employed to stimulate growth on later date cuttings have included: 1) 75-watt bulbs for a night interruption from 10:00 to 2:00 until budbreak occurs, and 2) fertilization with 200 mg/l 20-20-20 once a week (Drew, et al., 1993). For most species, the night interruption treatment resulted in maximizing overwinter survival. In addition to stimulating new shoot growth, the propagator should root the cuttings into containers or else leave them in an unheated greenhouse overwinter to insure good survival. Disturbance of the fragile new root systems can have a negative impact on winter survival rates.

For *Q. robur*, the direct incorporation of Osmocote fertilizer (15-11-13) into a peat/sand/perlite medium (1:1:1) at a rate of 2.0 g/l has been shown to result in higher rooting and bud break percentages (Spethman and Harms, 1993). Successful examples of oak species that have been reported to root from softwood cuttings include *Q. acutissima*, *Q. coccinea*, *Q. palustris*, *Q. phellos*, *Q. petraea*, *Q. lyrata*, *Q. robur*, *Q. rubra*, *Q. shumardii*, and *Q. virginiana*.

### C. Micropropagation

There have been successful examples of oak propagation by various tissue culture techniques. Auxiliary shoot multiplication in vitro has been reported for such species as: *Q. alba*, *Q. rubra*, *Q. robur*, *Q. petraea*, *Q. shumardii*, *Q. lobata*, *Q. suber*, *Q. acutissima*, and *Q. serrata*.

In most cases, cultures have been derived from embryo explant material, rather than from mature trees. However, successful techniques have been developed in Europe for *Q. robur* and *Q. petraea* by utilizing stump sprouts (Chalupa, 1993).

Since oaks are somewhat amenable to auxiliary shoot multiplication techniques, the question then centers on rooting. Ex vitro rooting is much cheaper, since the invitro step is avoided and both acclimation and rooting can occur at the same time. Besides *Q. petraea*, limited success was achieved for *Q. robur* using genetically improved seedorigin material in Germany (Meier-Dinkel, et al., 1993).

It is important to consider, in any discussion of micropropagation technology, how tissue cultured propagules grow in comparison to seedling origin trees. In one example, Chalupa (1993) stated that, for *Q. petraea* at least, tissue cultured oaks derived from seeds exhibited similar appearance and growth characteristics as seedlings after 8 years in the field.

While somatic embryogenesis techniques have great potential for oaks, and some positive results have been realized, more work is needed to perfect the protocols required to produce repeatable results for the genus (Jorgensen, 1993).

### Summary

The propagation of *Quercus* species by various techniques remains a dynamic field of study for both researchers and practitioners. While the refinement of the procedures attempted to date will continue, it is important to recognize the biological fact that the genus can be manipulated positively in most cases. Traditional seed propagation techniques have been developed to produce economically large numbers of seedlings that are capable of high survival rates in the field. Vegetative propagation of selected individuals is feasible through the use of grafting and softwood cutting technologies. Tissue culture protocols are being developed at a rapid rate in several laboratories, and hold great promise for the future propagation of superior trees for both forestry and horticultural end products.

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*Quercus stellata*. Ancient Trees, Tulsa, Oklahoma, USA. © Guy & Edith Sternberg.