Preliminary Progress on the Asexual Propagation of Oaks

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INTRODUCTION

There are many techniques propagators use to induce adventitious root formation on softwood cuttings. The application of auxins and the manipulation of stockplant juvenility and light, however, have proven to be powerful factors in the battle to asexually propagate difficult-to-root woody species by cuttings. The use of root-promoting hormones such as IBA is the most common treatment given to these recalcitrant species; however, the effects of juvenility and light have not been fully exploited as treatments in the cutting bench. Juvenility is the use of explant material taken from the biologically juvenile portions of the stock plant. In a number of species, juvenile tissue is the only tissue which will generate adventitious roots. As an example of the importance of physiological age versus chronological age, cuttings of Douglas fir were taken from 26-year-old trees. Cuttings taken from the bottom third of the trees rooted 71% while those from the top third rooted 52% (Roberts and Moeller, 1978). Although there has been no single explanation for this occurrence, there have been some common theories expressed as to the relationship between juvenility and adventitious root initiation. Stem anatomy, levels of endogenous rooting co-factors, and presence of preformed root initials are factors most commonly associated with the effect (Clark, 1982).

In *Hedera helix* for example, the stems of mature tissue contain a ring of sclerenchyma fibers that some have suggested may be a physical barrier to root development (Clark, 1982). Cuttings taken from juvenile portions of the plant have fewer of the thick, lignified sclereids and fibers, thus making it easier for the elongating root primordia to penetrate the periderm (Davies, 1984). Juvenile shoots of English ivy also appear to possess increased levels of rooting co-factors, while mature tissue of the species contains higher levels of rooting inhibitors (Clark, 1982). Preformed root initials are also abundant in the juvenile shoots of English ivy, while the mature stem portions are completely lacking any such characteristics (Clark, 1982). Regardless of a physiological explanation for increased adventitious root formation in juvenile stem tissue, its effect is indisputable, and is considered one of the most important and most overlooked factors in propagation by cuttings.

A third technique propagators will often consider when trying to propagate difficult-to-root species is that of etiolation. Etiolation as a pretreatment to cutting propagation involves excluding light from stock plants as the new shoot emerges from the dormant bud. Before these shoots are gradually acclimated to normal light conditions, a black Velcro™ band (approx. 2 cm diameter) can be placed at the base of the new shoot, which will become the cutting base, enabling that portion of the stem to remain in an etiolated state. The anatomical effects of etiolation can be compared to those of juvenility. Herman and Hess (1963) studied the effect of etiolation on *Phaseolus vulgaris* L. and *Hibiscus rosa-sinensis* L. They found that etiolated shoots possessed some root primordia in the stem while the light-grown plants did not. They also found the cells of etiolated plants were less lignified, with

thinner walls and less cellular differentiation within the stem tissue. The etiolated tissue was more sensitive to auxin treatments, and contained higher concentrations of endogenous auxin, compared to light-grown tissue. All of these effects should hasten the process of adventitious rooting either by aiding the development of root primordia or facilitating their easier emergence. Maynard and Bassuk (1987) experimented extensively with etiolation and banding and have been successful in increasing rooting percentages of a variety of species using this technique. Four-year-old seedlings of *Castanea mollissima* did not root at all when grown in light conditions, with or without the blanching effect of an applied band. The etiolated plants, however, rooted 44% without a band, and 100% with a band applied. Likewise, *Quercus coccinea* did not root under any treatment except the etiolated and banded treatment (46%) (Maynard and Bassuk, 1987).

Currently, most oaks used in the landscape are propagated by seed, with the resulting seedlings varying in traits (Drew and Dirr, 1989). Unfortunately, a good, quick method for asexual oak propagation has yet to be perfected. Tissue culture, although it has made great improvements recently (93% rooting of *Q. suber*), still takes well over 12 months with several subcultures every 4 weeks (Romano et al., 1992). This process would appear to be too expensive and labor intensive for the small nurseryman. Budding and grafting has only produced limited success (Hartmann, et al., 1990), while Drew and Dirr (1989) received moderate to poor success from various species using softwood and semihardwood cuttings.

OBJECTIVE

The objective is to develop an improved method for clonal propagation of *Quercus* species. Improving the rooting percentage of oaks would greatly aid research efforts that are trying to select for superior types to incorporate into the urban setting. Also a simple, effective propagation method would help the nursery industry in selecting those oak clones which show better pest resistance, branching habits, and fall color.

We manipulated stock plant juvenility and light levels during this project. We also employed the traditional, yet modified method of stooling. Our modification allowed new shoots which emerge from the cut back stock plant to be grown in complete darkness, compared to the traditional method of continually mounding around shoots as they grow in the light. Shoot tips were allowed to see light only after their bases had been mounded with soil following the initial etiolation phase. This would combine the acknowledged rejuvenating effect of stooling with etiolation at a crucial stage of stool shoot emergence.

Because propagation by cuttings is still the most cost-effective method (Davies and Hartmann, 1988), we wanted to see if this process would work for cuttings taken from etiolated seedlings. Here the shoots which emerge from the buds were totally etiolated for a short time as they grew to eventually be used as cuttings.

We looked at the effects of etiolated versus nonetiolated shoots, the effects of different IBA concentrations, and whether GA applications to force bud break affects rooting of cuttings.

MATERIALS AND METHODS

Stooling. Potted oaks 5 to 8 years old were brought into a 75F day/65F night greenhouse on 11 Jan., 1996 after they had received a 3-month-cold requirement at 36 to 38F. Just before bud break, the stems were removed just above the soil level

and half were placed under black cloth. Once etiolated shoots had elongated 10 to 20 cm, they were sprayed on their lower halves with 8000 ppm of IBA in two different carrier solvents, 50% aqueous ethanol and 20% aqueous DMSO.

A pot with the bottom cut out of it was placed over the top of the plant with its bottom resting on the soil. A light weight soilless potting mix was then filled in and around the etiolated shoots until they are completely covered. Shoots emerging from this soil were allowed to grow under normal light conditions. Eight weeks later, shoots were examined for root formation.

Cuttings. Acorns of *Q. bicolor* were sown in December 1995, and allowed to germinate and grow under normal greenhouse conditions. Once seedlings set bud they were sprayed with a solution of 500 ppm GA_3 dissolved in ethanol and water (15 : 85, v/v) in order to force subsequent bud break. Plants were sprayed every 3 days until bud swell. As buds began to swell, they were black clothed or grown in full light. When shoots elongated 10 to 15 cm, they were banded and slowly readjusted to light growing conditions over the period of 1 week. After 3 more weeks, shoots were removed just below the band, dipped in varying concentrations of IBA and stuck in a propagation bench under mist for rooting.

Dormant 1-year seedlings of various species were also brought into the greenhouse and forced to break bud. Just before bud break, they were black clothed and treated as the *Q. bicolor* seedlings above. Several oak species were used during the experiments, including, *Q. acutissima, Q. bicolor, Q. macrocorpa, Q. palustris, Q. robur,* and *Q. rubra.*

RESULTS AND DISCUSSION

***Quercus bicolor* Seedlings.** Buds enlarged and elongated approximately 1 week after the first application of GA_3 were used to induce a second flush of growth from which cuttings could be taken. Other researchers have shown an increased rooting percentage of cuttings treated with GA. Sagee, et al. (1990) applied a ring of 2.9 mM GA dissolved in a lanolin paste to the stems of hardened citrus shoots just before bud break. Cuttings were taken from the subsequent growth that emerged and were dipped in a 29.6 mM IBA rooting powder. Ninety percent rooting was achieved with this treatment compared to 19% rooting when shoots were treated with IBA alone. Ernstsen and Hansen (1986), however, found that when the apex of new cuttings of *Pinus sylvestris* were treated with an aqueous solution of GA_3 (2.0 mM) immediately following excision, rooting percentage was significantly decreased. In the current experiment we saw no significant difference between the etiolated (16.7% rooting) and the light grown shoots (9.3% rooting) (P=0.07). There was, however, a highly significant difference between the banded (17.6% rooting) and the unbanded (5.1% rooting) shoots (P=0.0025) (Table 1). This is very obvious with the light-grown plants, 0% rooting unbanded, compared to 27.8% rooting when the shoot was banded (Table 1). This may suggest that it is not solely the extent of etiolation during shoot development that influences adventitious rooting, but rather the etiolated state of the shoot at the time of excision that matters. It is no surprise that there was a significant difference between the 0 mM and the 19.7 and 39.4 mM IBA concentrations (P=0.005 and 0.03, respectively) (Table 1). Although the difference between the 0 mM and the 39.4 mM treatments was statistically significant, the lower rooting percent of the 39.4 mM may suggest that this concentration is super optimal to the

young shoots. However, it should also be noted that there is no significant difference between the 19.7 mM and the 39.4 mM IBA concentrations (P=0.17). Further research is needed to determine the optimal IBA concentration for cuttings of this species.

Table 1. Percent of shoots of *Quercus bicolor* which formed adventitious roots.

	Percent rooting				
	Etiolated		Light grown		
IBA (mM)	-B	+B	-B	+B	Total %
0	8.3[a]	11.1[a]	0.0	0.0	4.9
19.7	--	33.3[b]	0.0	27.8[b]	20.4
39.4	8.9[a]	16.7[a,b]	11.1[a]	16.7[ab]	13.2
Total %	8.3	20.4	3.7	14.8	

Treatments with the same letter were not significantly different at the P=0.05 level .
+B = banded; -B= unbanded.

Based on these results, we would expect the banded shoots treated with 19.7 mM IBA to root with the highest percentage. This, in fact, was the case with the etiolated plants. The etiolated, banded, and 19.7 mM IBA percent rooting (33%) is two times greater than either of the similar treatments (etiolated, banded, 0 mM — (11%), etiolated, banded, 39.4 mM — (16.7%), and over a standard error away. The light-grown plants were not quite so convincing. Although the light-grown, banded, 19.7 mM IBA-treated cuttings had the highest percent rooting (27.8%), it was not significantly higher than the 39.4 mM (16.7%). Based on these results, it appears that cutting propagation of *Q. bicolor* should be approached using the banding technique, with or without etiolation, and a quick dip of 19.7 mM IBA in 50% ethanol.

Future research should concentrate on reducing the instances of iron chlorosis in the stock plant. Based on current research in a separate experiment using *Quercus bicolor*, and a similar procedure, we expected much higher than the 33% rooting which was achieved here. Despite the weekly application of fertilizer with micronutrients and the foliar application of ferrous sulfate, nearly all of the shoots exhibited symptoms of iron chlorosis. The chlorosis in some of the shoots was so severe that they were completely white. A nutrient analysis of these white shoots revealed an iron concentration of 12 $\mu g\ g^{-1}$ dry weight compared to 276 $\mu g\ g^{-1}$ dry weight in a nonchlorotic shoot.

Rooting of Cuttings from 1-Year-Old Plants. *Quercus bicolor* rooted at much higher levels in this experiment, suggesting that iron chlorosis in the seedlings was a highly significant factor in depressing rooting percentage. Treatments of *Q. bicolor* plus *Q. macrocopa*, *Q. palustris*, and *Q. robur* consisted of etiolated and light-grown

Table 2. Percent rooting of shoots from 1-year-old stock plants of four *Quercus* species.

Species	Treatments +E+B+GA	+E+B-GA	+E-B+GA	+E-B-GA	-E+B+GA	-E+B-GA	-E-B+GA	-E-B-GA	Average
bicolor	90.9 (9)[A]	80.0 (13)	45.5 (15)	55.6 (17)	100.0 (0)	72.7 (14)	36.4 (15)	30.8 (13)	64.0
macrocarpa	88.9 (11)	50.0 (28)	40.0 (24)	40.0 (24)	50.0 (18)	0.0 (0)	0.0 (0.)	0.0 (0)	33.6
palustris	92.3 (7)	83.3 (11)	77.8 (14)	84.6 (10)	75.0 (13)	78.6 (11)	70.6 (11)	58.8 (12)	77.6
robur	50.0 (16)	61.5 (14)	7.1 (7)	0.0 (0)	29.7				
Average	70.4	56.4	54.4	60.1	75.0	50.4	26.8	22.4	

Abbreviations: +E = etiolated, +B = banded, +GA = sprayed with GA_3, -E = light grown, -B = no band applied, -GA = no GA_3.
Number in ( ) is standard error.

Table 3. Rooting percentage of greenhouse grown oak stoolbeds.

	Etiolated					
	DMSO			Ethanol		
	Shoots rooted/total	Percent	Std. error	Shoots rooted/total	Percent	Std. error
Q. acutissima	1/1	100.0	0	3/24	12.5	6.8
Q. bicolor	14/31	45.2	8.7	3/24	12.5	6.8
Q. macrocarpa	5/7	71.4	17.1	6/10	60.0	15.5
Q. palustris	3/3	100.0	0	1/2	50.0	35.4
Q. robur	13/14	92.9	6.9	9/24	37.5	9.9
Q. rubra	1/4	25.0	53.5	3/6	50.0	20.4
	37/60	61.7	6.3	22/66	31.9	5.6

	Light grown			
	DMSO		Ethanol	
	Shoots rooted/total	Percent error	Shoots rooted/total	Percent error
Q. acutissima	0/24	0	0/25	0
Q. bicolor	0/14	0	0/9	0
Q. macrocarpa	0/4	0	-	0
Q. palustris	0/30	0	0/28	0
Q. robur	0/4	0	0/7	0
Q. rubra	0/76	0	0/69	0

stockplants both of which were banded or not banded when shoots reached 5 to 7 cm. Before bud break half the stockplant buds were sprayed with 500 ppm GA_3 at 3-day intervals until bud break occurred. All cuttings received an IBA quick dip at 8000 ppm.

Again, *Q.bicolor* showed a consistently positive effect of banding without a clearly beneficial effect of either GA_3 or etiolation (Table 2). With *Q. macrocorpa,* etiolation plus banding plus GA_3 gave the highest rooting percentage (89%) while two out of the three treatments showed an intermediate effect. Shoots from light-grown plants without GA_3, regardless of banding showed no rooting.

Q. palustris showed reasonably good rooting with all treatments; the sole poorly rooting treatment occurring in the light-grown, unbanded and no GA_3 group. Even with only four of the eight treatments, *Q. robur* showed a clearly beneficial effect of etiolation.

Stoolbed Results. The modified greenhouse-grown stoolbeds showed some intriguing possibilities that warrant further research. Although previous researchers have shown no benefit from using stooling to propagate oaks, our modification using etiolated shoots and more penetrating carriers of IBA appears to have much promise. As the buds began to swell on the cut back plants, half were put under black cloth and half were light grown. When shoots reached 10 to 20 cm half of the shoots of each light treatments were sprayed on their lower halves with 8000 ppm IBA dissolved in 20% DMSO and the other half in 50% aqueous ethanol prior to mounding with soilless medium. Shoots were uncovered and rated for rooting after 8 weeks. None of the light-grown stool shoots rooted regardless of IBA carrier treatments (Table 3). The pre-etiolated shoots showed some impressive rooting. Of the species with reasonable replication, *Q. bicolor, Q. macrocarpa*, and *Q. robur*, rooting percentages in the DMSO treatment were 45.2%, 71.4%, and 92.9%, respectively (Table 3).

The ethanol treatment was lower in most all cases. Taking all the species together, DMSO increased rooting about 100% from 31% with ethanol to 61% with DMSO.

Further work examining the results of manipulating juvenility, IBA and light levels in oak propagation are being planned.

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DAVE BAKKER: What is the concentration of your DMSO?

NINA BASSUK: 20%.

CHARLES HEUSER: What was your survival?

NINA BASSUK: About 75% but we are not concentrating on survival at this time.