TECHNIQUES FOR ROOTING JUVENILE SOFTWOOD CUTTINGS OF NORTHERN RED OAK[1]

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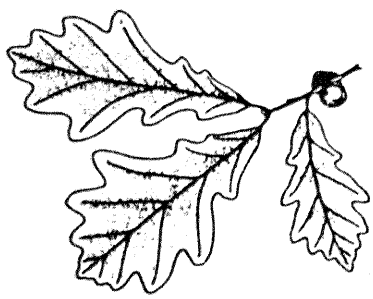

ABSTRACT.--Reliable methods for vegetative propagation of oaks are needed because of the problems and uncertainties associated with propagation from seed. An experiment was conducted to determine the effects of two indole-butyric acid (IBA) application methods (i.e., alcohol dip and talc) and light restriction techniques (i.e., etiolation and blanching) on rooting of softwood cuttings of juvenile northern red oak (Quercus rubra L.). The percent of rooting, number of roots, length of longest root, and root and stem dry weights were assessed after 6 weeks of intermittent mist in a greenhouse bench. Rooting occurred with all treatments, but IBA applications produced more roots per cutting. The method of IBA application, however, yielded significantly different results when used in combination with etiolation or blanching. Light restriction techniques enhanced both the number and size of resultant roots. The "best" combination in terms of number and size of roots was the quick-dip of 0.5% IBA in 95% EtOH and blanching. Age of cutting material is an important factor in rooting northern red oak.

INTRODUCTION

Reliable methods for vegetatively propagating oaks are needed because of the numerous problems and uncertainties associated with propagating oaks from seed. Some examples of these problems include variable acorn crops from tree to tree, species to species, location to location, and year to year (Olson and Boyce 1971), a high incidence of insect-damaged acorns (USDA Forest Service 1974), and numerous difficulties related to storing acorns (Suszka and Tylkowski 1980). Moreover, tree improvement with oak has not been practical because of the long time required for oaks to reach seed-bearing age and the difficulties with controlled pollination of trees with large seeds (Irgens-Moller 1955).

Effective methods for vegetative propagation of oak would insure dependable sources of material from year to year, avoid long juvenile periods, facilitate rapid propagation of clones with superior traits, and reduce plant variability for research purposes. Oak is considered difficult to propagate and few investigators have been able to achieve 75% or greater success in rooting oak (Hartman and Kester 1983). Some notable exceptions have been Thimann and Delisle (1939) with red oak hardwood cuttings, Farmer (1965) with juvenile cuttings of cherrybark oak, Hare (1977) with epicormic sprouts from the crown of water oak, and Cornu et al. (1977) with softwood cuttings from stump sprouts of English oak.

English oaks (white oak subgenus) are routinely propagated vegetatively from stump sprouts (Riedacker and Belgrand 1983) and softwood cuttings from mature trees (Smith and Schwabe 1984), but there are major differences in rootability between the white oak and red oak groups (Skinner 1953). To our knowledge no reliable techniques for rooting northern red oak (NRO) softwood or hardwood cuttings exist. We have not been successful in rooting NRO hardwood cuttings using the techniques of Thimann and Delisle.

Our preliminary tests using indole-butyric acid (IBA) (Cornu et al. 1977) in combination with light restriction techniques employed by horticulturists

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for propagating fruit trees (Farmer 1971; Howard 1981; 1983) show promise for rooting softwood cuttings from NRO stock plants and stump sprouts. The objective of this experiment was to determine the effects of two specific IBA application methods used in combination with two light restriction techniques on rooting softwood cuttings of juvenile NRO.

MATERIALS AND METHODS

Acorns from a known NRO (Quercus rubra L.) source located in Oneida County, Wisconsin, were collected in September 1983, float-tested in water, stored at -1°C for 6 months in 4 ml polyethylene bags, and stratified at 3°C for 3 months in a cold room. Six hundred acorns were pregerminated in July, 1984, and planted in individual 0.6-liter pots containing a 1:1 peat/sand mix, and grown in a greenhouse until epicotyl emergence (fig. 1). In August, half the seedlings were moved to a dark growth room at 24°C for etiolation (i.e., growth in dark lacking chlorophyll); the other half were grown in a controlled environment chamber set for 16-hour days, 27°/21°C temperature and photon flux density of 290 u mol $m^{-2}s^{-1}$. After 1 week the seedlings (i.e., average height: etiolated = 12 cm, light grown = 7 cm) were transferred to a greenhouse bench (25°C) equipped with overhead shade cloth. At this time, one-half of the etiolated and one-half of the light-grown seedlings were blanched (Howard 1983) using black latex tubing to restrict light at the basal 6.5 cm of stem, i.e., the rooting zone. After 1 additional week in the greenhouse, softwood cuttings were taken from all seedlings (age = 3 weeks from planting), and then divided into 5 equal-sized groups that received 5 different chemical applications (C_1 through C_5, fig. 1).

The application methods were: C_1) 0.5% IBA in 95% EtOH quick-dip, C_2) 0.5% IBA in talc powder (w/15% benomyl) after 5 min soak in $1g.l^{-1}$ boric acid, C_3) 95% EtOH quick-dip, C_4) talc powder (w/15% benomyl) after 5 min soak in boric acid and, C_5) no chemical application. The use of the boric acid soak in conjunction with the IBA-talc treatment enhances the absorption of IBA by the stem and thus increases rooting potential (Antoine Kremer, pers. communication). In all, the cuttings received a factorial series of treatments (fig. 1) that included 2 etiolation treatments (+,-), 2 blanching treatments (+,-), and the 5 application methods mentioned for a total of 20 treatments. Three replications of 10 each were then stuck in individual 350 ml pots in a 1:1 peat/sand mix, and randomly placed in a greenhouse mist bench (27°/21°C) equipped with automatic watering (5s/10 min). After 6 weeks, the percent of rooted cuttings, the number of roots ≥ 1 mm in diameter, length of longest root (mm), root dry weight (mg), and dry weight of the 6.5 cm stem rooting zone (mg) were determined for the various treatments. Statistical analysis was with ANOVA for a completely randomized design (Table 1). Orthogonal contrasts (Snedocor and Cochran 1967) were used to compare the chemical application methods (Table 2). Rooting percent data were transformed to arcsin $\sqrt{\%}$.

TABLE 1.--Analysis of variance table for northern red oak rooting experiment with degrees of freedom for each level. Comparisons presented in Table 2 are linear contrasts among the chemical applications.

Source	df
Treatments	19
A (Etiolation)	1
B (Blanching)	1
C (Chemical applications)	4
AB	1
AC	4
BC	4
ABC	4

RESULTS AND DISCUSSION

Rooting Percent

Rooting occurred in all factorial treatment combinations including the control and there were no statistical differences among the factorial combinations. The average rooting percent for the entire experiment averaged 88 $\pm$ 2% ($\bar{x}\pm$SE) and treatments ranged from 60 to 100%; even 87% of the control cuttings (no etiolation, no blanching, and no chemical application) rooted. One-hundred percent of the cuttings rooted in 2 of the 20 treatments; light-grown cuttings with blanching and IBA-95% EtOH quick-dip; and light-grown cuttings with blanching and no chemical applications (fig. 1), i.e., light-grown with blanching was common to both treatments.

Apparently, the young age of these softwood cuttings was an important factor in obtaining rooting because our previous attempts at rooting more lignified oak cuttings have been much less successful. Although the effect of age of the cutting varies with species (Hartmann and Kester 1983), apparently cuttings from young oaks are more readily rooted than those from older material (Komissarov 1964, Farmer 1965). Commercial applications of rooting softwood cuttings of oak trees, therefore, will probably depend upon either maintaining or reinstating juvenility in stock plants (Leakey 1983).

Number of Roots

Although the rooting percent did not vary statistically among the treatment combinations, the number of roots ≥ 1 mm in diameter did. The number of roots may even be more significant than rooting percentage as an indicator of eventual plantlet survival and establishment. Etiolation and blanching alone had no effect on the number of roots ($P>.05$), but both the type of IBA application method ($P=.001$) and the interaction of blanching and IBA application method had a significant effect ($P=.005$). The average number of roots per cutting in the whole experiment was 4.3 $\pm$ 0.9, and the range over the factorial treatment combinations

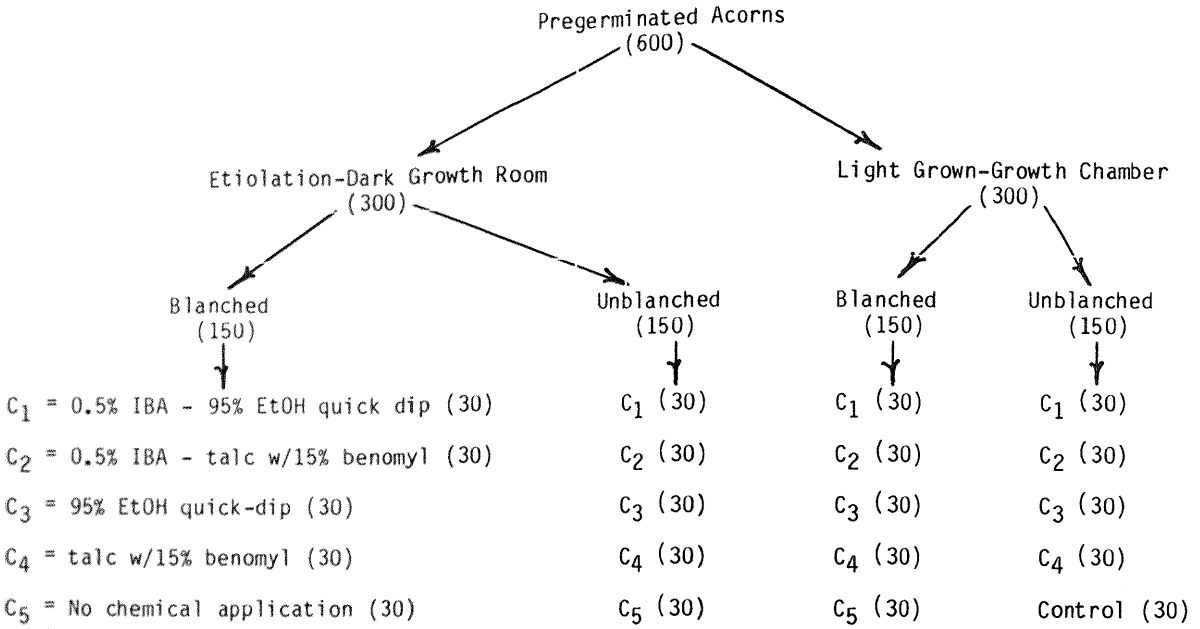

FIGURE 1.--Schematic of treatments used for rooting of softwood northern red oak cuttings. The number of cuttings in each treatment is in parenthesis; (30) represents 3 replications of 10 cuttings each. The control is a cutting receiving no etiolation, no blanching and no chemical application.

TABLE 2.--Linear contrasts for five chemical application methods used to root softwood cuttings of northern red oak.

Contrasts[1/]	Rooting Variable - Statistical Significance[2/]			
	# Roots	Longest Root	Root Dry Weight	Rooting Zone Dry Weight
C_5 vs. C_1, C_2, C_3, and C_4[2/]	**	n.s.	n.s.	**
C_1 and C_2 vs. C_3 and C_4	**	*	n.s.	**
C_1 vs. C_2	n.s.	**	**	**
C_3 vs. C_4	n.s.	*	*	n.s.

1/Linear contrasts of Snedocor and Cochran (1967); C_1) 0.5% IBA - 95% EtOH quick-dip, C_2) 0.5% IBA in talc (w/15% benomyl), C_3) 95% EtOH dip, C_4) talc w/15% benomyl, and C_5) no chemical application

2/** = Significant at 0.01 probability level; * = significant at 0.05 probability level; n.s. = non-significant

averaged from 2.0 to 8.8 roots per cutting. The highest number of roots was with no etiolation, with blanching and IBA 95% EtOH quick-dip; the lowest number was with the combination of etiolation, blanching and no chemical application. Even the control yielded 3.1 roots per cutting. The distribution of roots on the rooting zone of the cutting also varied with IBA application method, although this distribution was not determined quantitatively in this experiment. The IBA-EtOH quick-dip resulted in more roots at the cutting base, while IBA-talc applications gave roots that were often distributed in vertical rows on the cutting. This may be related to the location of vascular bundles in the stems and the physical differences in application of the rooting hormone; but regardless, the anatomical and physiological details of this phenomenon need to be studied further.

Clearly, cuttings treated with IBA had significantly more roots than those that did not receive hormone applications. But there was no difference in the number of roots whether hormone

was administered by dipping or in talc (Table 2). However, there was a pronounced difference in number of roots with the method of application when used in combination with blanching. With blanching, the IBA quick-dip yielded significantly more roots per cutting ($\bar{x}$=8.1 roots/cutting, n=60) than those grown in the light ($\bar{x}$=5.3). By contrast, the IBA-talc method yielded an equally significant increase in the number of roots in the light ($\bar{x}$=7.0) when compared to the blanched cuttings ($\bar{x}$=4.3). It should be noted that the same interaction was not observed for etiolation and IBA treatment method as was for the blanched cuttings. Thus, etiolation and blanching do not act in a similar fashion in terms of root formation on cuttings. Although we have no direct evidence from our study, apparently IBA quick-dip succeeds in mobilizing carbohydrate to the rooting zone in the absence of light better than does IBA in talc. On the other hand, in the light IBA in talc seems to provide a slow release hormone concentration over time. Haissig (1984) discusses the subject of carbohydrate accumulation and rooting and has shown a positive relation between rooting and total carbohydrate accumulation in jack pine cuttings.

Length of Longest Root

After 6 weeks the length of the longest root averaged 99 $\pm$ 14 mm over the whole experiment and the range averaged from 57 to 135 mm. Although no significant effect of etiolation or blanching alone was observed (P=.08), there was a significant effect of IBA application method on root length (P=.003). IBA in talc in all cases resulted in shorter roots (Table 3). In fact, IBA in talc and talc alone resulted in shorter roots than the control. Either of the light restriction techniques (i.e., etiolation or blanching) used alone, or in combination with one another resulted in longer roots than when no light restriction was used (Table 4). Thus, it appears that IBA in 95% EtOH quick-dip used in combination with light restriction results in longer roots as well as more roots.

Root Dry Weight

Root dry weight is, of course, closely related to the number of roots and their size. The average root dry weight per cutting for the whole experiment was 32 $\pm$ 7 mg, the range averaged from 13 to 52 mg, and root dry weight of a control cutting was 23 mg. Root dry weight closely paralleled the root length data throughout (Tables 2 and 3). Again, as with the longest root, the IBA talc method resulted in the lowest root dry weights (Table 3). However, in the case of root dry weight the IBA-EtOH quick-dip was not significantly different from the EtOH dip. In addition, both EtOH dip applications had greater root dry weights than the other application methods (Table 3). This observation suggests that the alcohol itself may in some way be stimulating rooting in oak softwood cuttings.

Although the effects of etiolation and blanching were not as dramatic as the IBA treatments, higher average root length and root dry weight did occur when cuttings were subjected to light restriction (Table 4). Using etiolation and blanching together did not result in higher root dry weights, and of the two, etiolation usually resulted in higher root dry weights. Apparently, some form of light restriction (but not both) used in combination with IBA-EtOH quick-dip will result in the highest root dry weights. More information on the mechanisms of how these treatments effect carbohydrate accumulation and rooting in cuttings is clearly needed.

Dry Weight of Rooting Zone

Dry weight of the rooting zone is a variable that is often used in rooting studies to assess carbohydrate accumulations in the rooting zone. However, in this study, this variable is perhaps more difficult to interpret than in some experiments because of the extreme effect of etiolation on stem form and size. Etiolation resulted in thinner and more elongated stems (fig. 2A) when compared to light grown seedlings. For example, the root zone in cuttings with etiolation and no chemical application averaged 66 mg compared to 114 mg in cuttings without etiolation and no chemical application. The average root zone weight for the whole experiment was 98 mg.

The results for dry weight of the rooting zone were essentially the opposite of the root dry weight indicating that root growth is probably a result of mobilization of carbohydrates from the stem (Table 3). For example, IBA dip applications were significantly different than IBA talc applications (Table 2), but in just the opposite direction of root dry weight. That is, the IBA talc applications were low in root dry weight and high in rooting zone dry weight (Table 3). It should be noted that even though the initial dry weight of rooting zone in etiolated cuttings was low, they still produced large numbers of roots and large root dry weights when treated with IBA-EtOH quick-dips. This observation suggests that a substantial mobilization of carbohydrate must have occurred from above the rooting zone to accomodate the numerous expanding root growth centers (Haissig 1984). Fundamental experiments including detailed carbohydrate and hormone analysis will be needed to properly understand this phenomenom.

CONCLUSIONS AND RECOMMENDATIONS

As Farmer (1974) suggested, the lack of reliable rooting techniques for oaks is probably due to insufficient research rather than inherent species difficulties. Softwood cuttings of 3-week-old juvenile northern red oak can be rooted effectively in peat/sand media with IBA applications and intermittent misting in a greenhouse bench (fig. 2A). Light restriction techniques such as etiolation and blanching enhance the number and size of resultant roots, but both techniques are not necessary. The "best" IBA application method, however, varies with the type of light restriction technique chosen. Our results show that juvenile NRO can be successfully rooted using blanching for

TABLE 3.--Average root variables of softwood cuttings of northern red oak for five chemical application methods.

Application Method	Root Variables[1/]			
	# Roots	Longest Root (mm)	Root Dry Weight (mg)	Rooting Zone Dry Weight (mg)
C_1: 0.5% IBA in 95% EtOH quick-dip	6.7	104	40	98
C_2: 0.5% IBA in talc w/15% benomyl	5.6	76	22	115
C_3: 95% EtOH quick dip	2.9	119	38	91
C_4: Talc w/15% benomyl	3.4	94	27	98
C_5: No chemical application	3.1	100	31	88

[1/] Averaged over experiment - each value represents average of 120 cuttings (e.g., C_1 is pooled over experiment). Standard errors of the difference between 2 averages are: a) # roots = 0.7, b) longest root = 10, c) root dry weight = 5, and d) rooting zone dry weight = 4.

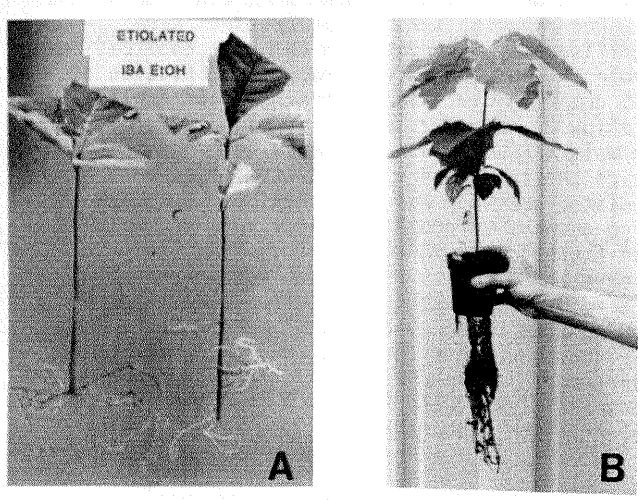

FIGURE 2.--Rooted softwood cuttings of northern red oak. A. Cutting rooted using etiolation and 0.5% IBA-EtOH quick-dip. B. Two-flush softwood cutting of northern red oak after rooting and subsequent acclimation to ambient conditions in a controlled environment chamber.

1 week prior to treating cuttings with a 5 min soak in $1g.l^{-1}$ of boric acid, followed by a quick dip in 0.5% IBA in 95% EtOH. Even though in this experiment we took softwood cutting material from only the initial flush of growth, we have had some success using these methods for rooting cuttings from older stock plants. Farmer (1965) also had success in rooting the initial flush of cherry-bark oak using lower concentrations of IBA, but his success declined with age of the stock plant. Our higher IBA concentrations and use of light restriction represent distinct differences from Farmer's technique. Etiolation can also be used for rooting NRO, but from a practical standpoint, we think blanching is probably most easily administered. Etiolation may be more practical for propagating oak *in vitro* culture than with conventional vegetative propagation. If for some reason light restriction is not possible, NRO cuttings can be rooted using IBA in talc or alcohol.

We have had some preliminary success in acclimating newly rooted NRO cuttings to the ambient environment for eventual outplanting (fig. 2B). Rooted cuttings are removed from the mist and hardened in a humidified plastic tent in a controlled environment chamber. Those plants are repotted after the 2nd or 3rd flush is completed. There is some evidence that etiolated seedlings may perform better than light grown seedlings in field plantations (Hopper *et al*. 1983).

The experiments reported here are a first step in being able to vegetatively propagate red oak. Future studies will focus on rooting materials that will allow multiple reproduction of clones. Possible techniques include using hedges (Hartmann and Kester 1983), forcing mature branches with plant-growth regulators (Larson 1960; Schoeneweiss 1963), inducing epicormic sprouting, using softwood cuttings from the canopy of mature trees, and rooting stump sprouts. Etiolation experiments may also provide a useful experimental approach for

studying the fundamental aspects of root anatomy and carbohydrate relations which are needed to better understand the rooting of oak.

TABLE 4.--Comparison of root dry weight (g) and largest root (mm) among light restriction treatments, i.e., etiolation, blanching, and no light restriction. Values represent means, n=150.

	Root Dry Weight (g)	
	Etiolated	Light Grown
Blanched	30.7	37.6
Unblanched	32.2	25.5
	Longest Root (mm)	
	Etiolated	Light Grown
Blanched	98	108
Unblanched	102	88

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