STAGE OF SHOOT DEVELOPMENT AND CONCENTRATION OF APPLIED HORMONE AFFECT ROOTING OF NORTHERN RED OAK SOFTWOOD CUTTINGS

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Abstract.--Reliable techniques for vegetative propagation of northern red oak (NRO) are needed for physiological studies and tree-improvement programs because of the inherent variability of seedlings and the difficulty of rooting mature tissue. The objective of our study was to evaluate the effects of stage of shoot development and increased IBA concentration on rooting percent, number of roots, and root dry weights of softwood cuttings from NRO seedlings. Three concentrations of IBA (0.5%, 1.0%, and 1.5%) and blanching (light restriction with black rubber tubing), were evaluated for the above rooting variables on softwood cuttings taken from the terminal shoots of two- and three-flush NRO seedlings. The highest IBA concentration studied (1.5%) gave the highest rooting percent. Blanching at full leaf expansion, followed by hormone application 4 days later, produced the highest rooting in two-flush oaks. Similar trends were found in the number of roots and root dry-weight. However, rooting still declined with the age of the stock plant.

Additional keywords: Quercus rubra L., indole-butyric acid, vegetative propagation, blanching, Quercus morphological index (QMI), tree improvement.

Reliable techniques for vegetative propagation of northern red oak (Quercus rubra L.) are needed to achieve plant uniformity for morphological and physiological research, and for propagation of elite genotypes in tree improvement programs. Genetic variation inherent in open pollinated families limits the use of seedlings in physiological studies. Moreover, oak tree-improvement efforts have been limited because of the problems with collecting and storing acorns, the lengthy period needed for most oaks to reach seed-bearing age, and the difficulty in pollinating oaks (Irgens-Moller 1955, Beineke 1979). Clonal propagation of superior northern red oak (NRO) phenotypes would improve planting stock and produce genetically uniform material for research studies.

Oak is considered difficult to propagate and few investigators have been able to achieve commercial success of 70% or greater in rooting oak (Hartman and Kester 1983). In general, cuttings from young trees are more easily rooted than those from older trees (Farmer 1965, Duncan and Mathews 1969, Borzan et al. 1983). Probably the most significant improvements in rooting oak have come from advances in rooting hormones and in the

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techniques used in their application. Cutting propagation for Quercus petraea and Q. robur have already been developed to an applicable scale in Germany (Kleinschmit 1986). In a previous study, Isebrands and Crow (1985) had little difficulty rooting one-flush NRO; but in subsequent trials rooting declined for second- and third-flush softwood cuttings. In these trials, cuttings of two- and three-flush oaks were all taken at approximately the same arbitrary morphological and physiological stages of shoot development. However, Spethmann (1982) has suggested that oak cuttings must be taken at a specific physiological stage in order to obtain rooting. Therefore, we investigated whether the stage of shoot development and concentration of applied hormone affects the rooting of softwood cuttings. This information is needed to develop a technique for vegetatively propagating mature superior NRO.

The results of three separate yet related experiments are reported here. The specific objectives were to determine if: A) age of cuttings (one-, two-, or three-flush) taken from NRO affects rooting; B) the concentration of an exogenous hormone affects rooting; and C) the stage of development within a flush affects rooting. The three experiments will be hereafter referred to as: Exp A - flush study, Exp B - hormone concentration study, and Exp C - stage-of-development study.

PROCEDURES

Acorns from a single open-pollinated NRO tree growing in Oneida County, Wisconsin were used for all three experiments. Seedlings were grown in 1:1 peat:sand mix, fertilized once a week with a modified Hoagland's solution, and watered as needed. Environmental conditions were set at 27-21°C day-night temperatures with a 16-hr photoperiod (Hanson et al. 1986).

In Exp A, the "best" hormone treatments from a previous rooting study (Isebrands and Crow 1985) were evaluated for rooting of softwood cuttings from one-, two-, and three-flush NRO seedlings. One of the following six treatments (Table 1, Exp A) was applied to 15 cm apical cuttings from plants at each of the three flushes. The treatments were: A1) a quick-dip application of 0.5% indole-butyric acid (IBA) in 95% ethanol (EtOH) preceded by blanching for 7 days, a technique that eliminates light to the rooting zone (the area of the stem where the IBA is applied and where the roots are to develop). A2) quick-dip application in 95% EtOH preceded by blanching; A3) no chemical application preceded by blanching; A4) 0.5% IBA in talc (with 15% benomyl[2]/) preceded by a 5 min soak in 1 $g \cdot l^{-1}$ boric acid; A5) talc (with 15% benomyl) preceded by a 5 min soak in the boric acid solution, and A6) no application, i.e., control. When present, leaves were removed from the the rooting zone and the remaining leaves were cut back to about 1/3 their original size. Three replications of ten softwood

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[2] Mention of trade names does not constitute an endorsement of the products by the USDA Forest Service.

cuttings for each of three flushes were treated; each cutting was inserted in a separate 350 ml pot with a 1:1 peat:sand mix, and then randomly placed on a mist bench with bottom heat at 27°C. Intermittent mist was applied at 5 sec bursts every 10 min in a glasshouse under 55% shade cloth. The percentage of rooted cuttings, the number of roots longer than 1 cm, and root dry-weight were determined for the various treatments after 60 days. Statistical analysis was with ANOVA for a complete randomized design. Rooting percentage data were transformed to arcsin (the square root of %). Orthogonal contrasts were used to compare the treatments (Snedocor and Cochran 1967). Because of the difficulty in rooting NRO and the variability of the material, the 0.10 probability level was used to test contrasts.

Table 1.--Treatment applications for experiments A, B and C

Experiment A	Experiment B	Experiment C
Blanched	Blanched	Blanched
A1-0.5% IBA in EtOH	B1-0.5% IBA in EtOH	C1-0.8% IBA Pre-lag
A2-EtOH	B2-1.0% IBA in EtOH	C2-0.8% IBA Lag
A3-No chemical	B3-1.5% IBA in EtOH	C3-0.8% IBA Post-lag
Not Blanched	B4-No chemical	C4-95% EtOH Pre-lag
A4-0.5% IBA in talc	Not Blanched	C5-95% EtOH Lag
A5-talc	B5-0.5% IBA in EtOH	C6-95% EtOH Post-lag
A6-No chemical	B6-1.0% IBA in EtOH	
	B7-1.5% IBA in EtOH	
	B8-No chemical	

In Exp B, four concentrations of IBA (0%, 0.5%, 1.0%, and 1.5%, W:W) in 95% EtOH were applied as a quick-dip. Half the cuttings were blanched for 7 days and half were not blanched giving a total of eight treatments (Table 1, Exp B). Three replications of ten cuttings for each of the second and third flushes were treated. The cuttings were inserted in separate pots with 1:1 peat:sand mix and placed on a mist bench in a polyethylene greenhouse. The mist was controlled by a Mist-A-Matic misting device adjusted to keep the surface of the leaves wet throughout the 60-day rooting period. Rooting was analyzed as in Exp A.

In Exp C, two-flush NRO were grown to one of three developmental stages based on a *Quercus* morphological index (QMI) (Hanson et al. 1986). The seedlings were grown in a polyethylene greenhouse with environmental conditions, mix, containers, and nutrients similar to those described above.

When the developing leaves of the second flush attained a horizontal position, each seedling was assigned a specific stage of development (pre-lag, lag, or post-lag) for treatment (Table 1, Exp C). The lag stage as defined by Hanson et al. (1986) starts when the second leaf from the top of a flush is fully expanded. The seedlings were blanched 4 days before they reached the specific stage and were then treated with either 95% EtOH or 0.8% IBA in 95% EtOH. Blanching times were estimated as closely as possible based on the development of the individual seedlings. Three replications of ten cuttings per treatment were used as in the other experiments. The cuttings were maintained throughout the rooting period, and the results analyzed as in Exp A.

RESULTS AND DISCUSSION

Experiment A

In the flush study, there were significant differences in rooting percentages among flushes and among treatments but no significant interaction between the flushes and treatments. All first-flush cuttings except the control rooted at 70% or higher (range 73-100%) (Table 2).

Table 2.--Flush Study (Exp A): Treatment means (± S.E.) for rooting variables of one-, two-, and three-flush NRO softwood cuttings.

Treatment	Rooting %	Number of roots	Root Dry Weight (mg)
1st FLUSH			
Blanched			
A1-0.5% IBA in EtOH	100 ± 0	14.4 ± 1.7	210 ± 41
A2-95% EtOH	90 ± 10	3.0 ± 0.1	79 ± 2
A3-No Chemical	83 ± 12	3.4 ± 0.7	124 ± 22
Not Blanched			
A4-0.5% IBA in Talc	93 ± 7	7.8 ± 0.7	140 ± 11
A5-Talc	73 ± 3	2.5 ± 0.5	44 ± 9
A6-No Chemical	53 ± 13	1.7 ± 0.4	51 ± 13
2nd FLUSH			
Blanched			
A1-0.5% IBA in EtOH	57 ± 23	2.2 ± 1.0	51 ± 22
A2-95% EtOH	70 ± 6	1.7 ± 0.6	152 ± 103
A3-No Chemical	27 ± 9	0.8 ± 0.3	16 ± 3
Not Blanched			
A4-0.5% IBA in Talc	20 ± 6	0.5 ± 0.3	8 ± 6
A5-Talc	10 ± 6	0.2 ± 0.1	4 ± 3
A6-No Chemical	13 ± 7	0.5 ± 0.3	6 ± 3
3rd FLUSH			
Blanched			
A1-0.5% IBA in EtOH	47 ± 9	1.6 ± 0.6	41 ± 10
A2-95% EtOH	13 ± 9	0.3 ± 0.3	2 ± 2
A3-No Chemical	7 ± 3	0.1 ± 0.0	6 ± 6
Not Blanched			
A4-0.5% IBA in Talc	3 ± 3	0.2 ± 0.2	2 ± 2
A5-Talc	3 ± 3	0.1 ± 0.1	1 ± 1
A6-No Chemical	3 ± 3	0.1 ± 0.0	1 ± 1
ALL THREE FLUSHES			
Blanched			
A1-0.5 IBA in EtOH	68 ± 11	6.1 ± 2.2	100 ± 30
A2-95% EtOH	58 ± 12	1.7 ± 0.4	78 ± 37
A3-No Chemical	39 ± 12	1.4 ± 0.6	49 ± 20
Not Blanched			
A4-0.5% IBA in Talc	39 ± 14	2.8 ± 1.3	50 ± 23
A5-Talc	29 ± 11	1.0 ± 0.4	16 ± 7.5
A6-No Chemical	23 ± 9	0.7 ± 0.3	20 ± 8.8

However, rooting percentage declined in the second and third flushes (range 10-57% and 3-47%, respectively). The highest rooting percentage for the first and third flushes and for the average of all three flushes was found with the blanched treatment and 0.5% IBA in EtOH. However, the quick-dip in 95% EtOH gave the highest rooting percentage for two-flush cuttings. Apparently, ethanol alone stimulates some root formation, but, as the age of the seedling increases, hormone application (IBA application) becomes increasingly important.

There were significant differences among flushes and treatments in the number of roots and the root dry-weights, and there were also significant interactions between flush and treatment. This suggests that treatment may vary with the age of the material. The marked differences of higher rootability in the more juvenile material (first-flush over second-flush over third-flush) was also evident in both the number of roots and root dry-weight. Even though all of our material was juvenile, but from different flushes, these results are consistent with previous reports that mature oaks are more difficult to root than juvenile material (Komissarov 1964, Farmer 1965, Isebrands and Crow 1985).

Blanching significantly increased rooting percentage, number of roots, and root dry-weight in all flushes (Table 3), and this improvement became more pronounced with cuttings from each subsequent flush. Rooting percentage was above 80% in all blanched treatments for the first flush, which again demonstrates the ease of rooting one-flush material. When IBA was applied in conjunction with blanching, the number of roots and root dry-weights were significantly higher for the first and third flushes than when no IBA was applied. These results indicate that light restriction can be used to improve rooting.

Table 3.--Flush Study (Exp A): Orthogonal linear contrasts for six treatments used to root softwood cuttings of northern red oak

	1st FLUSH Rooting			2nd FLUSH Rooting			3rd FLUSH Rooting		
Contrast	%	#	Dry Weight (mg)	%	#	Dry Weight (mg)	%	#	Dry Weight (mg)
Blanched vs. Not Blanched	**	**	**	**	**	**	**	**	**
Blanched: IBA vs. No IBA	NS	**	**	NS	NS	NS	**	**	**
Not blanched: IBA vs. No IBA	**	**	**	NS	NS	NS	NS	NS	NS
Blanched: EtOH vs. No EtOH	NS	NS	NS	**	NS	*	NS	NS	NS
Not Blanched: Talc vs. No Talc	NS	NS	NS	NS	NS	NS	NS	NS	NS

** = Significant at 0.05 probability level
* = Significant at the 0.10 probability level
NS = Not significant

Application of IBA with blanching did not significantly increase rooting percentage for the first flush because blanched cuttings without IRA also rooted well. This trend is supported by the findings of

Isebrands and Crow (1985) on the rooting of one-flush NRO. However, IBA does promote significantly more roots and higher root dry-weights, even in first-flush cuttings. There are no significant differences in rooting percentage, number of roots, and root dry-weights for blanched material of the second flush when comparing the IBA treatments versus treatments without IBA. Perhaps this is because ethanol by itself promoted rooting in juvenile material. However, all three rooting variables were significantly higher for IBA treatments versus no IBA treatment in the third flush, indicating the importance of hormone treatment for rooting this tissue. Talc alone had no significant effect.

Experiment B

In the IBA concentration experiment, there were significant differences between flushes and among treatments for rooting percentage and the number of roots. However, there were only significant differences among treatments and not between flushes for root dry-weight. Therefore, both flushes were pooled for analysis of root dry-weights. No significant interaction was found between flushes and treatments for all rooting variables. For blanched two-flush material the rooting percentage and number of roots increased with increasing IBA concentration to 83% rooting and 3.1 roots per cutting for 1.5% IBA (Table 4). Unblanched material generally rooted at a lower rate than blanched. This result reinforces the importance of blanching in conjunction with hormone application. Rooting decreased more in the third flush than in the second flush and increased with IBA concentration. The values for the rooting variables for the blanched 1.0% IBA treatment of the third flush appear to be unexplainably low and are not consistent with trends. These low values could mask the statistical significance of some treatments. The greatest root dry-weight was for blanched material treated with 1.5% IBA, although there were no significant differences among concentrations of IBA in both blanched and unblanched material.

Table 4.--Hormone concentration study (Exp B): Treatment means (+ S.E.) for rooting variables of two- and three-flush NRO cuttings.

Treatment	2nd Flush % Rooting	2nd Flush # Roots	3rd Flush % Rooting	3rd Flush # Roots	Pooled 2nd & 3rd Flush Root Dry Weight (mg)
Blanched					
B1-0.5% IBA	60 ± 12	1.8 ± 0.7	50 ± 8	1.0 ± 0.2	125 ± 47
B2-1.0% IBA	77 ± 3	2.8 ± 1.1	33 ± 3	0.7 ± 0.2	80 ± 27
B3-1.5% IBA	83 ± 12	3.1 ± 0.1	60 ± 8	2.0 ± 0.4	130 ± 19
B4-No Chemical	43 ± 9	1.1 ± 0.4	23 ± 3	0.4 ± 0.1	25 ± 8
Not Blanched					
B5-0.5% IBA	67 ± 3	1.7 ± 0.3	43 ± 9	1.0 ± 0.2	64 ± 11
B6-1.0% IBA	57 ± 18	1.8 ± 0.5	57 ± 7	1.5 ± 0.7	108 ± 31
B7-1.5% IBA	57 ± 13	1.6 ± 0.4	63 ± 7	1.9 ± 0.4	82 ± 15
B8-No Chemical	30 ± 17	0.7 ± 0.5	23 ± 9	0.5 ± 0.2	22 ± 10

Blanching alone significantly increased the rooting of two-flush but not three-flush material. This phenomenon is probably a result of low rooting values for the 1.0% IBA blanched treatment of the third flush. Application of IBA in conjunction with blanching significantly increased the rooting for both flushes. The application of IBA on unblanched material significantly increased rooting for both flushes except for the number of roots of two-flush cuttings. However, the number of roots was significant at the 12% probability level. These results illustrate the importance of IBA application in rooting of NRO softwood cuttings. Chong (1980) rooted English oak (Quercus robur 'Fastigiata') with 2.0% IBA. Increasing the IBA concentration above 1.5% may increase rooting of the more mature NRO material.

Experiment C

IBA application significantly increased rooting for all developmental stages. This result is clearly illustrated by the means of the rooting percentages. Rooting with IBA treatments ranged from 60 to 70% and without IBA ranged from 33 to 53% (Table 5). Although the differences in rooting percentage for pre-lag and post-lag cuttings (60 and 70%, respectively) were not significantly different, the post-lag treatment had significantly greater root dry-weights. This finding again shows the importance of IBA application to rooting. Although the IBA did not significantly increase rooting percentage, it did significantly increase the mass of the root system. The insignificant differences can probably be attributed to our small sample size (only 30 individuals per treatment) and to the high standard errors. In general, treating the cutting with IBA at a later stage of development apparently is the "best" method for rooting NRO softwood cuttings.

Table 5.--Stage-of-development study (Exp C): Treatment means (+ S.E.) for rooting variables of different aged cuttings.

Treatment	Rooting %	# of Roots	Root Dry Weight (mg)
C1 0.8% IBA - Pre-lag	60 ± 10	2.0 ± 0.0	113 ± 20
C2 0.8% IBA - Lag	63 ± 9	1.9 ± 0.3	100 ± 19
C3 0.8% IBA - Post-lag	70 ± 12	2.7 ± 0.9	256 ± 87
C4 Control Pre-lag	33 ± 7	1.3 ± 0.6	61 ± 26
C5 Control Lag	53 ± 9	1.0 ± 0.3	117 ± 41
C6 Control Post-lag	53 ± 13	1.4 ± 0.5	170 ± 49

CONCLUSIONS

Juvenile one- and two-flush NRO cuttings can be easily rooted at or above commercially acceptable levels using established rooting techniques. This conclusion is based on consistent results obtained on two-flush material in all three experiments. However, as the material matures, rooting becomes more difficult and new techniques need to be developed. Increased IBA concentrations, possibly as high as 2.0%, and aryl esters of

IBA have proven effective with other species (Haissig 1983, Struve and Arnold 1986) and need to be tested on NRO. In addition, blanching significantly improved rooting. Vegetative propagation of superior northern red oak phenotypes will require further advances in rooting techniques.

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