# Cutting Propagation of Grafted Mature and Juvenile Northern Red Oak

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A rooting trial evaluated the rooting success of cuttings from mature and juvenile, grafted and ungrafted northern red oak (NRO). Buds from seedlings and from 4 mature NRO trees were grafted onto juvenile and mature rootstock. Shoot cuttings were collected from the grafts and directly from seedling and mature trees and subjected to a rooting trial. Of all treatments, cuttings from juvenile material rooted best. However, the rooting of cuttings from mature trees was also relatively successful. Percentage rooting of cuttings was significantly related to ortet genotype and ontogeny and was not directly influenced by grafting. The number of roots per cutting and post-rooting flushing behavior was significantly related to ortet ontogeny. Juvenile rootstock had little effect on the rooting, number of roots per cutting, flushing behavior, and overwintering success of cuttings from mature NRO. Mature rootstock negatively influenced the number of roots per cutting, flushing behavior, and overwintering success of shoots from grafted juvenile buds.

# INTRODUCTION

Northern red oak (*Quercus rubra* L.) is a widespread and abundant species important in both traditional and urban forestry. It is a genetically diverse species and therefore offers great potential for tree improvement. Vegetative propagation, especially from mature individuals, is difficult and a severe hinderance to research and to the full utilization of the genetic variation within this species. The objective of this research was to identify techniques for the successful vegetative propagation of ontogenetically mature northern red oak (NRO).

#### MATERIALS AND METHODS

**Mature Plant Material.** Four seed-producing NRO trees provided sources of mature plant material for grafting and cuttings. In June 1991, the trees were 12, 13, 19, and 22 m tall and 29, 39, 68, 73 cm in dbh for ortets 1, 2, 3, and 4, respectively. Cuttings developed *in situ* from the mature ortets were designated M1, M2, M3, and M4.

**Grafting Treatments.** In April of 1990, dormant scion wood was collected from the lower 1/3 of the crown of the mature trees. One hundred dormant buds from each ortet were bud-grafted (spring T-budded) onto 1-year-old potted NRO rootstock.

These 1990 grafts are referred to as 90Xs (X = genotypes 1, 2, 3, 4). The successful  $1990 \, grafts \, (47\%)$  were maintained throughout the growing season and overwintered in an unheated greenhouse. In 1991, these grafts were used as a source of dormant buds for grafting and as a source of cuttings for the 1991 rooting trial.

In April 1991, ca. 100 dormant buds were collected from the 1990 grafts and budded onto 1-year-old potted NRO rootstock. Cuttings from these serial-grafted plants are referred to as 9190Xs, (X = genotype number 1, 2, 3, or 4). In addition, approximately 100 dormant buds collected directly from each of the four mature trees (genotypes 1, 2, 3, 4) and from 1-year-old potted seedlings (J) were grafted onto 1-year-old potted NRO rootstock. Cuttings from these single series grafted plants are referred to as 91Xs (X = 1, 2, 3, 4, and J).

In April and May 1991, additional grafts using approximately 100 buds from each of 1-year-old seedlings and mature tree #2 were made onto the lower 1/3 of the crown of mature tree #1. Cuttings for these treatments are referred to as J-M1 and M2-M1, respectively.

In May 1991, grafted rootstocks were decapitated just above the grafts to stimulate the grafted buds to break dormancy. Decapitation at this time provided synchrony of budbreak for both the indoor grafts and outdoor *in situ* ortets. At the same time, the 1990 grafts (90Xs) were brought into the heated greenhouse to stimulate budbreak.

**Juvenile Plant Material**. Pre-stratified acorns were sown in pots during April 1991 in a greenhouse and grown to provide 2nd-flush cuttings (J2) for use in the rooting trial. In May 1991, acorns from the same seedlot were similarly sown in pots in a greenhouse to provide 1st-flush cuttings (J1) for use in the rooting trial. Additionally, cuttings were collected from shoots that arose from along the 1st flush of decapitated 1-year-old seedlings (JDs).

Rooting Procedures. Mature tree cuttings (MXs) were collected from the lower 1/3 of the crown. Cuttings were collected daily and kept cool and moist until processing during the same day. All leaves were removed from cuttings, except three at the apex. The basal end of each cutting was freshly trimmed and dipped in 1.2% w/w IBA and ethanol for 5 sec and allowed to dry for 1 min. While drying, the remaining leaves were trimmed perpendicular to the midvein to about 1/2 of their original size. Cuttings were inserted into predibbled holes in moist media (1 perlite: 1 peat: 1 coarse white sand) in 115 cc Ray Leach Super Stubby Cells and lightly watered prior to placement in the rooting chamber. The rooting chamber was a polyethylene tent located in a greenhouse. Intermittent fog was provided by four ultrasonic humidifiers (Sunbeam model 667).

In both years, benomyl (Benlate at 2.4 g liter<sup>-1</sup>) was sprayed on the leaves every month during the rooting period. Cuttings were checked for rooting and number of roots per cutting 80 days after sticking. Rooting success was defined as the presence of at least one root at least 5 mm in length. The number of roots per cutting reflects the number of roots >5 mm in length originating from the stem or callus of a cutting. Cuttings that had not rooted after 80 days were placed back into the high humidity chamber and checked again 40 days later. Data summaries reflect the total cuttings rooted over 120 days.

In 1991, 80 days after sticking, those cuttings that had rooted were potted into 6.5-  $\rm cm^2$  pots by 23-cm-tall pots filled with Pro-Mix BX and set into a shaded acclimation

tent. For the next 50 days, daylength was supplemented with 18 h day<sup>-1</sup> of artificial light from sodium vapor lamps. Humidity was initially maintained at 100% and gradually decreased over 20 days to ambient greenhouse levels, at which time the shade was removed. Late-rooting cuttings were acclimated for 10 days. The rooted cuttings were transferred to an unheated greenhouse for overwintering.

#### **RESULTS**

**Percentage Rooting.** Rooting averaged 72.2% over all treatments but there were large differences among the treatments ranging from 96% for J1 to 20% for M3 cuttings (Table 1). Significant genotypic and ontogenetic effects were present but grafting was not significantly related to rooting.

**Genotypic Effects.** Rooting was dependent on genotype for mature ungrafted cuttings (chi-square, P<0.001). Logit analysis indicated that there was a significant relationship between scion genotype and rooting (P=0.491).

Grafting Effects. Cuttings from grafts of the mature genotypes rooted more often than their ungrafted counterparts (69% vs 59%). However, when considering genotypes, logit analysis revealed that rooting success was not significantly related to the main effects of grafting or genotype (P<0.05). Averaged over genotypes, percent rooting was 56, 59, 70, and 79 for 91Xs, mature, 9190Xs, and 90Xs, respectively. Grafting did decrease the differences between genotypes but not significantly so. Only for #3, the ortet with the poorest rooting performance, did every grafting treatment increase rooting compared to ungrafted controls (from 20 to 58%). Grafting treatments variably affected the other genotypes.

Ontogenetic Effects. Chi-square analysis revealed a significant relationship between rooting success and cutting maturity when comparing all mature cuttings as a group versus all juvenile cuttings (P<0.001). Rooting of 2-month-old J1 cuttings (96%) was considerably greater than mature cuttings (59%). Interestingly, rooting of only slightly older 3-month-old seedlings (J2) dropped significantly to 82% (chi-square, P=0.05) and was less than one of the four mature genotypes. Therefore, chronological age of the plant was not the determining factor of rooting success. This was further evidenced by the 94% rooting success of the 1-year-old JD cuttings.

Number of Roots Per Rooted Cutting. There were significant differences among the treatments for the number of roots per cutting (P<0.0001). Rooted cuttings from juvenile seedlings had significantly more roots per cutting than those from mature trees (Table 1). Grafting treatments using juvenile rootstock did not significantly influence the number of roots per cutting. Based on the number of roots per rooted cutting, there is no evidence of grafting-induced rejuvenation.

Cuttings from juvenile buds that were grafted onto a mature tree (J-M1) had significantly fewer roots (3.0) compared to cuttings from juvenile seedlings (23.3, 14.9, 14.3 for JD, J2 and J1, respectively) and to cuttings from juvenile buds grafted onto juvenile rootstock (13.5 for 91J). There apparently was an influence of the mature rootstock on the number of roots per rooted cutting but not on rooting success, suggesting that these measures of juvenility were independently controlled.

Overwinter Survival. By June 1992, 63% of the 1991 rooted cuttings were alive. Differences in overwinter survival closely mirrored rooting success (Table 1). For the

Table 1. Percentage rooting, roots/cutting, and overwinter survival by treatment for northern red oak cuttings.

Cutting type	Number of cuttings (n)	Rooting (%)	No. Roots per cutting	Overwinter survival (%)
M1	50	56.0	2.0	40.7
<b>M</b> 2	50	86.0	4.1	79.4
<b>M</b> 3	50	20.0	1.4	20.0
M4	50	74.0	5.8	47.2
J1	50	96.0	14.3	95.8
J2	49	81.6	14.9	80.0
JD	51	94.1	23.4	93.6
901	28	71.4	3.8	60.0
902	76	89.5	6.8	70.3
903	35	71.4	3.2	80.0
904	38	81.6	6.8	77.4
911	39	51.3	3.0	45.0
912	41	70.7	4.9	51.7
913	15	46.7	1.6	28.6
914	37	54.1	4.6	57.9
91J	42	90.5	13.5	70.3
91901	27	81.5	2.8	50.0
91902	44	72.7	4.5	65.6
91903	30	43.3	7.3	54.5
91904	30	83.3	3.6	52.2
J-M1	15	93.3	3.0	50.0
M2-M1	11	81.8	5.7	44.4

MXs = mature trees, genotypes 1,2,3,4.

J1, J2 = one- and two-flush seedlings, approximately 2 and 3 months old, respectively.

JD = 1st flush of one-year-old formerly multiflush but decapitated (clipped off) seedlings.

<sup>90</sup>Xs = 1990 grafts, 1-year-old rootstock, X=genotypes 1,2,3,4.

<sup>91</sup>Xs = 1991 grafts, 1-year-old rootstock, X=genotypes 1,2,3,4 and J (juvenile).

<sup>9190</sup>Xs = serial grafted on 1-year-old rootstock (1991 and 1990).

J-M1 = juvenile buds grafted on mature tree #1.

M2-M1 = mature genotype #2 buds grafted on mature tree #1.

most part, those treatments that had high rooting success (Js), overwintered well (90% survival) and those that had poor rooting success (M3), had poor overwinter survival (20%).

Overwinter survival was related to rooting date. For cuttings that had rooted by 80 days, 70% survived, compared to those that rooted after 80 days, only 25% survived. Of all juvenile treatments that had rooted, 97% had done so within 80 days. Only 62% of all treatments using ortet #3 had rooted by that time. Perhaps the cuttings that were early rooters were able to store more photosynthate and harden-off more completely than the late rooters. Post-rooting flushing behavior and the number of roots per cutting did not appear to directly influence overwinter survival as within-treatment averages for dead and surviving rooted cuttings were similar. Although the actual cause(s) of overwinter mortality was not certain, the roots of many of the dead rooted cuttings were infected with *Phytophthora*.

## DISCUSSION

Cuttings of mature NRO are difficult to vegetatively propagate. However, in this study, rooting success of cuttings from mature NRO was 59%. The high level of rooting success does not appear to be an anomaly as additional rooting experiments performed in the same chamber in subsequent years achieved similar high rooting success. Even with relatively high rooting success, large differences in responses were apparent among treatments. For cuttings from mature trees, significant genotypic effects were found for rooting and overwintering success. Even after grafting, which decreased the differences in rooting, significant genotypic effects persisted.

Rooting and overwintering survival decreased significantly in a relatively short time when comparing 2-month-old (J1) and 3-month-old (J2) seedlings. However, chronological age was not a good predictor of success as the first flush of older (1-year-old) JD seedlings that were decapitated did not further decline but rooted and survived at levels similar to J1 seedlings. Ontogenetic effects are somewhat obscured by the ease of rooting and high overwintering percentages of cuttings from the much older and much larger M2 tree. The number of roots per rooted cutting was strongly related to donor plant ontogeny regardless of whether or not it had been grafted onto juvenile rootstock.

Juvenile rootstock had no significant main effect on rooting success, the number of roots per rooted cutting, or overwinter survival. For these responses, shoot system differences (genotypic and ontogenetic) were maintained. Cuttings from juvenile buds grafted onto mature rootstock rooted in percentages similar to those grafted onto juvenile rootstock but had fewer roots per cutting, and lower overwintering success. Mature rootstocks were apparently inhibitory for two of the measured characteristics, but not all three. There were also differences between J1 and J2 cuttings in rooting, flushing, and overwintering success but not for the numbers of roots per rooted cutting. This suggests that the responses are under relatively independent control.

The relatively small influence of juvenile rootstock on mature meristems does not appear to be due to ontogenetic rejuvenation but possibly physiological invigoration of the resulting shoots. For the most part, meristems appear to have predetermined rooting responses regardless of grafting. Assuming the differences between juvenile and mature shoots for rooting success are predetermined in buds, then expansion

of preformed buds originally set on a mature tree, whether it occurs in situ or grafted onto juvenile rootstock, should result in shoots that perform similarly. It follows that if ontogenetic rejuvenation of a meristem were to occur as influenced by juvenile rootstock, it must happen during the time of bud formation when the primordia for the next flush are formed. In our study, the corresponding treatments would be the 90Xs and the 9190Xs for which cuttings came from buds that formed on juvenile rootstock. The different environment provided by the juvenile rootstock during bud formation apparently did not change the mature character of the meristems and, for the most part, the cuttings responded similarly to their grafted (91Xs) or ungrafted counterparts.

The presence of significant interactive effects suggest that if ontogenetic rejuvenation is possible, it may be genotypically dependent. It is also possible that it may take additional grafting phases for more definitive indications of rejuvenation to be manifested.