Micro-Positional Differences in Cutting Origin Influence Propagation of *Quercus rubra*

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Grafting and rooting trials followed shoot formation and rooting ability of individual buds from a mature northern red oak. Sixty-four matched pairs of dormant twigs were chosen with a total of 701 buds identified by position along each parent twig. Apical bud clusters and individual lateral buds from one randomly selected twig of each pair were grafted onto potted nothern red oak seedlings and stimulated to develop shoots. Buds from the paired counterparts were left on the tree (in situ) to develop normally.

In situ, shoots developed from 100%, 75% and 18% of terminal buds (Position I), lateral buds within the distal 1/3 of the twig (Position II), and lateral buds within the proximal 2/3 of the twig (Position III), respectively. When grafted, shoots developed from 39%, 54%, and 42% of the buds from the same positions, respectively. Therefore, shoots from grafted and in situ buds originated from two positionally different bud populations. All shoots were subjected to a rooting trial. Overall, cuttings from grafts had greater rooting (48%) than cuttings developed in situ (14%). However, for grafted and in situ bud pairs which both produced shoots, rooting success was similar. Rooting was not influenced by twig position or origin for in situ shoots. For cuttings from grafts, rooting was significantly related to position on the twig with 25%, 46%, and 67% success for Positions I, II, and III, respectively.

Though it is well known that rooting differs for shoots from widely varying crown positions, these results suggest that rooting differs on a much smaller scale, among buds along a twig. Results suggest that buds which normally develop into shoots in situ are not as competent to root (and perhaps more determined in their developmental fate) as those at more proximal portions of twigs that usually maintain dormancy in situ but form shoots when grafted. Although increased rooting through grafting is often attributed to physiological or ontogenetical rejuvenation by juvenile rootstock, these results suggest that grafting may serve to select for buds with high rooting potential by allowing their development into shoots and indirectly select against buds with low rooting potential through reduced shoot development.

INTRODUCTION

Inducing adventitious roots on cuttings of northern red oak (*Quercus rubra* L.) and many woody plant species is often attainable when the ortet is juvenile but exceedingly difficult when mature. Rooting success of tissues originating from mature plants has been shown to be enhanced if scions are first grafted onto seedling rootstocks, and cuttings are taken from the graft. Accordingly, it has been suggested that grafting rejuvenates mature tissues. However, the true effect of grafting remains unclear.

Prior grafting and rooting trials by the author have shown that bud-grafting mature northern red oak (NRO) onto seedlings produced cuttings with increased rooting and more juvenile-like leaf morphology, suggesting partial rejuvenation of mature tissues. However, these cuttings from mature grafts exhibited other mature characteristics such as low numbers of roots per cutting, limited flushing, and reduced post-rooting growth. Also, more horizontal or branch-like plagiotropic growth of rooted cuttings from grafts indicated that increased rooting in grafts may also be attributable to predetermined differences between axillary buds used in grafting and terminal buds that form most shoots that develop in situ.

Rooting can vary considerably for cuttings arising from widely differing crown positions of woody plants where maturation gradients (cyclophysis) may exist (Copes 1992; Ridding 1976). Black (1972) working with 24-year-old *Pseudotsuga menziesii* (Mirb.) Franco, did not find rooting differences due to cyclophysis, but did find topophytic (branch order) differences. Rooting differences in branch order were also found in *Sequoia sempervirens* (D. Don) Endl. plants that had been maintained in a hedged condition (Power et al., 1988). Presumably, variability in rooting success may occur on a smaller scale, such as among buds (meristems) along a twig.

Considering this, the effect of grafting on rooting may not be manifested through a physiologically or ontogenetically rejuvenating change of existing buds. Rather, grafting may increase rooting by allowing the development of a greater proportion of shoots from axillary buds that are inherently more rootable.

MATERIALS AND METHODS

In late Winter 1992, a mature open-grown seed-producing NRO tree 13 m tall and 39 cm in diameter at 1.3 m above the ground was chosen for the study. On the tree, 64 matched pairs of dormant twigs were chosen based on similar crown position, twig size, and number and type of buds/twig. For reference, a twig refers to the dormant shoot formed in the previous growing season that bears dormant terminal and axillary buds. A shoot refers to the leafy extension growth from buds that broke in Spring 1992. Shoots were used to collect cuttings for the rooting trial and are referred to as in situ shoots or graft shoots, respectively, according to whether they formed in place or after the buds were grafted onto seedling rootstocks. Overall, 701 buds were identified by parent twig and position along the twig. Twig length (TL) was measured (mm) from the base of the apical bud to the apical bud scale scar of the previous shoot growth extension. For each bud, the distance (mm) from the base of the apical bud (DA) was also recorded. The relative position (RP) of each bud was determined by DA/TL.

One randomly selected twig of each pair was selected to remain on the tree and the buds developed normally in situ. The twigs paired counterparts were removed from the tree and the buds were grafted onto potted NRO seedling rootstocks in a greenhouse in early April. For these twigs, all axillary buds positioned at least 5 mm below the apical bud were individually T-budded (Hartmann and Kester, 1983). Apical and closely subtending axillary buds within the 5 mm of apex were grafted as one scion using a side veneer graft (Hartmann and Kester, 1983). In all, 296 single buds and 64 apical scions were grafted. The grafting method for the apical scions did not allow for the development of the axillary buds in the distal 5 mm of the twigs. Because of this, the in situ counterparts in this region were excluded from the study.

Approximately 5 weeks after grafting, the seedling rootstocks were removed above the grafts in order to stimulate shoot development of the grafted buds in synchrony with those remaining on the tree. Each in situ and grafted bud was checked and shoot formation was recorded in early June. Shoots from one-half of the twig pairs were randomly chosen for use in another study. Remaining shoots were subjected to a rooting trial reported in this paper.

For the rooting trial, collections of softwood cuttings were made over consecutive days in mid June after shoots had fully expanded and prior to initiation of a new flush of growth. All leaves except three at the apex were removed from cuttings. The basal end of each cutting was freshly trimmed and dipped in a solution of 10,000 ppm 1H-indolebutyric acid (IBA) and ethanol for 5 sec and allowed to dry. While drying, the leaves were trimmed in half perpendicular to the midvein. Cuttings were inserted 3 cm deep into moist medium (perlite, peat, and coarse white sand [1 : 1 : 1, by volume]) in 115 cc Ray Leach Super Stubby Cells™ (Steuwe and Sons, Corvallis, Oregon). Cuttings were watered and randomly placed within the rooting chamber.

The rooting chamber consisted of separate 1-m-tall box-shaped frames of polyvinyl chloride pipe on three adjacent 1.7-m × 3.0-m roller benches. One sheet of clear 6-mil polyethylene covered all three frames, forming one large chamber. Intermittent fog was directed into the chamber by four ultrasonic humidifiers (Sunbeam model 667, Laurel, Mississippi) placed outside of the tent near the corners. Whitewash was applied on the greenhouse to reduce solar irradiance and heating inside the chamber. Benomyl (Benlate at 2.4 g liter^{-1}) was sprayed on the leaves at least once every month during the rooting trial. After 95 days, the cuttings were examined for root formation.

Data collection included the relative position of each bud along each twig, whether the buds formed a shoot in situ or after grafting, and if so, whether the shoots rooted in the rooting trial. First-year acorns were located in the approximate middle ⅓ section of nearly every twig (relative position 0.33 to 0.67 units). These developing acorns excluded graftable vegetative buds at these positions. Because of this natural division of vegetative buds along the twigs, results are summarized by three positions: the terminal (Position I), the distal ⅓ of the twig excluding the terminal (0.00 < relative position ≤ 0.33, or Position II), and the proximal ⅔ of the twig (0.33< relative position ≤ 1.00, or Position III).

Chi-square analysis was used to test for significant relationships between the treatments at the P=0.05 level (Roscoe, 1975). Specific pairwise comparisons in shoot production and rooting among bud position (I, II, III) and among bud treatment (grafted or in situ) were made at the P=0.05 level using CONTRAST a computer program (Hines and Sauer, 1989) based on a chi-square procedure described by Sauer and Williams (1989).

RESULTS

Shoot Production. Shoots formed from 59% of the buds left to develop in situ, and shoot development was dependent (P<0.001) on bud position of origin when considering all positions simultaneously. In situ, all Position I, most Position II, and a few Position III budsformed shoots (Table I). All specific pairwise comparisons (Positions I vs II, I vs. III, and II vs III) showed that the number of shoots differed

Table 1. A comparison of the percentage of buds producing shoots in situ or in response to grafting, by position of bud origin on the twig. See text for position designations.

Twig Position	Buds on in situ twigs (n)	Buds producing shoots in situ[1] (%)	Buds grafted (n)	Buds producing shoots when grafted[1] (%)	Comparing in situ and grafted shoot production by position[2]
Position I	64	100.0 a	64	39.1 y	*
Position II	166	74.7 b	138	54.3 x	*
Position III	130	17.7 c	139	42.4 xy	*
All Positions	360	58.6	341	46.6	*

[1] Shoot formation values in the same column with the same letter are not significantly different at the p=0.05 level based on pairwise comparisons using CONTRAST.

[2] Comparisons by position denoted with * are significantly different at p=0.05 using CONTRAST.

at all positions for buds developed in situ. However, when grafted, shoot production was somewhat reduced at 47% and was independent (P=0.055) of position when considering all positions simultaneously. When grafted, specific pairwise comparisons among positions showed that only Positions I vs II differed (Table I).

Comparing in situ and grafted treatments at each position, shoot production was greater (P<0.001) for in situ buds than grafted buds at Positions I and II. However, at Position III, shoot production was greater (P<0.001) for grafted buds compared to those buds left to develop in situ.

Rooting. Shoots formed from grafts rooted more (P<0.001) frequently (48%) than shoots formed in situ (14%) over all positions (Table II). However, considering only the 35 grafted and in situ bud pairs for which both buds of each pair had produced shoots, rooting success was marginally higher for graft shoots vs in situ shoots (43% vs 23%) but the difference was not statistically significant (P=0.068). For grafted buds that produced a shoot whose in situ bud counterpart failed to, rooting was 58% (n=19). For in situ buds that produced shoots whose grafted bud counterpart failed to produce a shoot, rooting was 11% (n=47).

For in situ shoots, rooting was not related (P=0.594) to bud position. However, for graft shoots, rooting was (P=0.044) related to position with 25%, 46%, and 67% of the shoots rooting at Positions I, II, and III, respectively (Table II). Rooting was higher for graft shoots vs in situ shoots at Position II and Position III (P<0.002 and P<0.001, respectively), but not at Position I (P=0.300).

DISCUSSION

When left to develop in situ, shoots formed mostly from the more distal buds, Positions I and II, as is typical of decurrent species with proleptic shoot development (Brown et al. 1967). Of the total number of shoots produced in situ, 30% originated from terminal meristems (Position I) only 11% were from the proximal ⅔ of the twig (Position III). However, when grafted and removed from the influence of apical control, buds originating formed shoots at more comparable percentages at all three positions. Therefore, shoots from Position I buds accounted for a smaller proportion (16%) and shoots from Position III buds accounted for a larger proportion (37%) of total shoots within the grafted population compared to the in situpopulation. Thus, shoots from grafts originated from a positionally different population of buds, generally less terminal and more proximal, than those formed in situ.

Rooting for shoots from grafts was greater than for those formed in situ, and the difference increased with increasing distance from the terminal. However, shoots of both treatments arose from different populations of buds. When considering a priori matched bud populations, where both matched bud pairs resulted in shoots, rooting success was not statistically greater for graft shoots.

Grafting allowed more shoot formation from buds originating from the proximal ⅔ of the twig than was the case in situ. These grafted Position III shoots had the highest rooting success (67%) compared to all other treatment combinations. Of the graft shoots, Position I shoots were the poorest rooters. There were also relatively few Position I shoots in the grafted population (n=12) compared to the in situ population (n=28) because grafting effectively eliminated many of these shoots (61%) by failing to form a successful graft union. In contrast, all Position I buds formed shoots in situ. Thus, in this study, the principal effect of grafting on rooting

Table 2. Adventitious rooting response of shoots formed in situ or after grafting by original bud position. See text for position designations.

Shoot cutting origin	Cuttings from in situ buds (n)	Rooting of in situ cuttings[1] (%)	Cuttings from grafts (n)	Rooting of grafted cuttings[1] (%)	Comparing rooting of cuttings originating in situ and from grafts by position[2]
Position I	28	10.7 a	12	25.0 x	ns
Position II	58	17.2 a	41	46.3 xy	*
Position III	11	9.1 a	18	66.7 y	*
All positions	97	14.4	71	47.8	*

[1] Rooting values in the same column with the same letter are not significantly different at the p=0.05 level based on pairwise comparisons using CONTRAST.

[2] Comparisons denoted with * are significantly different at p=0.05 using CONTRAST.

success may be viewed as a form of selection; selecting for buds that form shoots which root in high percentages and selecting against buds whose shoots root in low percentages.

Rooting was greater for shoots from Positions II and III when grafted, as compared to in situ, but not significantly so for Position I (terminal shoots). Also, where both members of matched bud pairs developed into shoots, rooting was no different. Thus, there was no evidence that grafting provided an exogenous effect to enhance rooting or rejuvenate individual meristems and their resulting shoots. Therefore, grafting's main effect in this study was to cause the preferential growth of shoots from meristems with an endogenous high capacity for adventitious root formation.

Thus for NRO the population of meristems with high adventitious root formation potential tends to remain dormant unless stimulated to grow by grafting. This is a more accurate description of the effect of grafting than "rejuvenation". Rooting can differ for shoots from different locations within the crown of the tree (Copes, 1992; Nautiyal et al., 1992; van den Driessche, 1983). Rooting also is affected by smaller scale branch order differences (Black, 1972; Power et al., 1988). Since crown architecture is the ultimate expression of differential development of terminal and axillary meristems, it is logical to conclude that rooting potential on a microscale (as shown in this study) is another expression of the same phenomenon at the macroscale.

If this is a general phenomenon, then other cultural treatments that increase rooting may also be due to meristem selection. This is especially true of hedging, or the severe and repeated pruning of stems from a plant, which has been shown to enhance rooting success in many species (Black, 1972; Copes, 1983; Copes, 1992; Howard et al., 1989; van den Driessche, 1983). Black and Copes have suggested that hedging stimulates rejuvenation or reinvigoration. However, Clark (1981) and Franclet (1983) suggested that the effect of hedging may be to induce the production of shoots which remain stable and juvenile and possess a high endogenous capacity for root formation. The results of this study support the latter interpretation. In other words, hedging may affect the overall rooting of a clone by reducing the proportion of terminal (Position I) and distally located axillary meristems that produce shoots which are poor rooters. And simultaneously, allowing a greater proportion of proximally located axillary meristems to form shoots (with high endogenous adventitious rooting capacity) that, if left unhedged, remain dormant. Similarly, this interpretation could be applied to serial propagation or even tissue culture treatments, which may serve to select out (epigenetically) difficult-to-root meristems from within a clone and concurrently select for, by their continued propagation, those which root readily.

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