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Clonal Propagation of *Quercus* Spp. Using a Container Layering Technique[1]

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Abstract

Clonal propagation of two oak species, *Quercus macrocarpa* Michx. and *Quercus bicolor* Willd. was accomplished using a modified container layering technique. The effects of gibberellin (GA_{4+7}) application on stock plant budbreak, as well as the effect of shoot position, stock plant age, and the role of indole butyric acid (IBA) solvents on rooting were investigated. Five hundred parts per million of GA_{4+7} applied once every fourth day increased budbreak in both *Q. macrocarpa* and *Q. bicolor* stock plants. Basal shoots of *Q. bicolor* from ontogenetically juvenile portions of the stock plant stem rooted 35.7% compared with 1.8% for shoots arising in more distal parts of the stock plant stem. In addition, stock plant age (2–6 years) had no effect on rooting. The effect of the various IBA solvents on rooting in *Q. bicolor* was 84.3% (98% ethanol), 81.3% (50% acetone), 100% (100% acetone), 28.6% (5% dimethyl sulphoxide-DMSO) and 65% in 10% DMSO respectively; all shoots in this experiment were treated with 10,000 ppm IBA in the respective solvents. IBA dissolved in DMSO treatments resulted in severe apical browning of shoots.

Index words: etiolation, juvenility, gibberellin, indole butyric acid, solvents.

Species used in this study: *Quercus macrocarpa* Michx., bur oak; *Q. bicolor* Willd., swamp white oak.

Chemicals used in this study: indolebutyric acid, (IBA); dimethyl sulphoxide, (DMSO); ethanol, acetone, Provide® (GA_{4+7}).

Significance to the Nursery Industry

The high degree of environmental adaptability and genetic variation that exists within *Quercus* Spp. makes genetic improvement through clonal selection highly desirable. Results from these experiments demonstrated that 500 ppm of Provide® (GA_{4+7}) applied every fourth day increased budbreak, which resulted in a greater number of treatable shoots in both *Q. bicolor* and *Q. macrocarpa* stock plants, using this container layering technique. Less toxic IBA solvents, such as acetone or ethanol, and the practice of severe cutting back of the stock plant were shown to improve rooting. In addition, the age of stock plants (2–6 years old) used with this technique did not decrease rooting in shoots, as has been observed in the rooting of shoots from plants of seedling origin (12). These identified practices improved rooting of shoots and demonstrate that the container layering technique is a means of propagating oaks asexually.

Introduction

Oak trees are used extensively in the landscape because of their longevity, toughness and beauty. They are wind pollinated (allogamous), fertile and highly heterozygous (7), hybridizing readily where two or more interfertile species occur (14, 17) and have aptly been described as 'notorious for their sexual infidelity' (18). This ability of oaks to hybridize readily has resulted in a number of hybrids with desirable landscape characteristics such as brilliant fall color, good structure, and branching habits as well as tolerance of drought and wet soil conditions. Unfortunately, very few selections exist due to the difficulty involved in clonally propagating this species by conventional methods like cutting propagation and grafting (7, 18). Recently, a modified container layering technique in conjunction with etiolation has been used for the propagation of this genus (8, 11).

To address the inconsistency and slow rate of budbreak observed from using this technique (personal observation), we conducted an experiment to evaluate the use of GA_{4+7} in increasing number of buds per cutback stump. Toxic effects observed from using DMSO, a more penetrating auxin solvent (5, 19), in the first four experiments presented in this paper prompted us to investigate the use of alternate IBA solvents like ethanol and acetone. Furthermore, to improve the practicality of this container layering technique, other stock plant treatments, such as the positional effect of shoots, stock plant age and the practice of stock plant cutback, on rooting were investigated.

Materials and Methods

Container layering technique. Dormant 2–6-year-old stock plants (of seedling origin) of both *Q. macrocarpa* and *Q. bicolor* potted into either #2, #3 or #5 pots (uniform except for experiment 4) were used. After receiving 3 months of chilling (December 12, 2001–March 12, 2002) at temperatures of 2.2C (36F), plants were brought into a warm 23.9C/18.3C day/night (75F/65F day/night) temperature greenhouse. All shoots were immediately removed from the stock plant leaving a 4 cm (~1.6 in) stem stump above the soil surface (Fig. 1 A). Upon evidence of bud swelling (small pink swellings) on the short stem base (Fig. 1 B), the containers were placed beneath black cloth tents (Fig. 1 C) and allowed to grow in near darkness (98% light exclusion, measured using a LI-189 portable light meter), until the shoots reached a desired height of 10–12 cm (~3.9–4.7 in). The tops of the black tents were draped with white plastic and fans placed beneath the tents to facilitate air circulation and prevent heat buildup. Shoot growth proceeded rapidly within one to two weeks, with characteristic pale colored etiolated stems and long internodes with unexpanded leaves. When a majority of shoots in a container reached the desired height of 10–12 cm (~3.9–4.7 in), the 4 cm (~1.6 in) base (from the soil surface) of each

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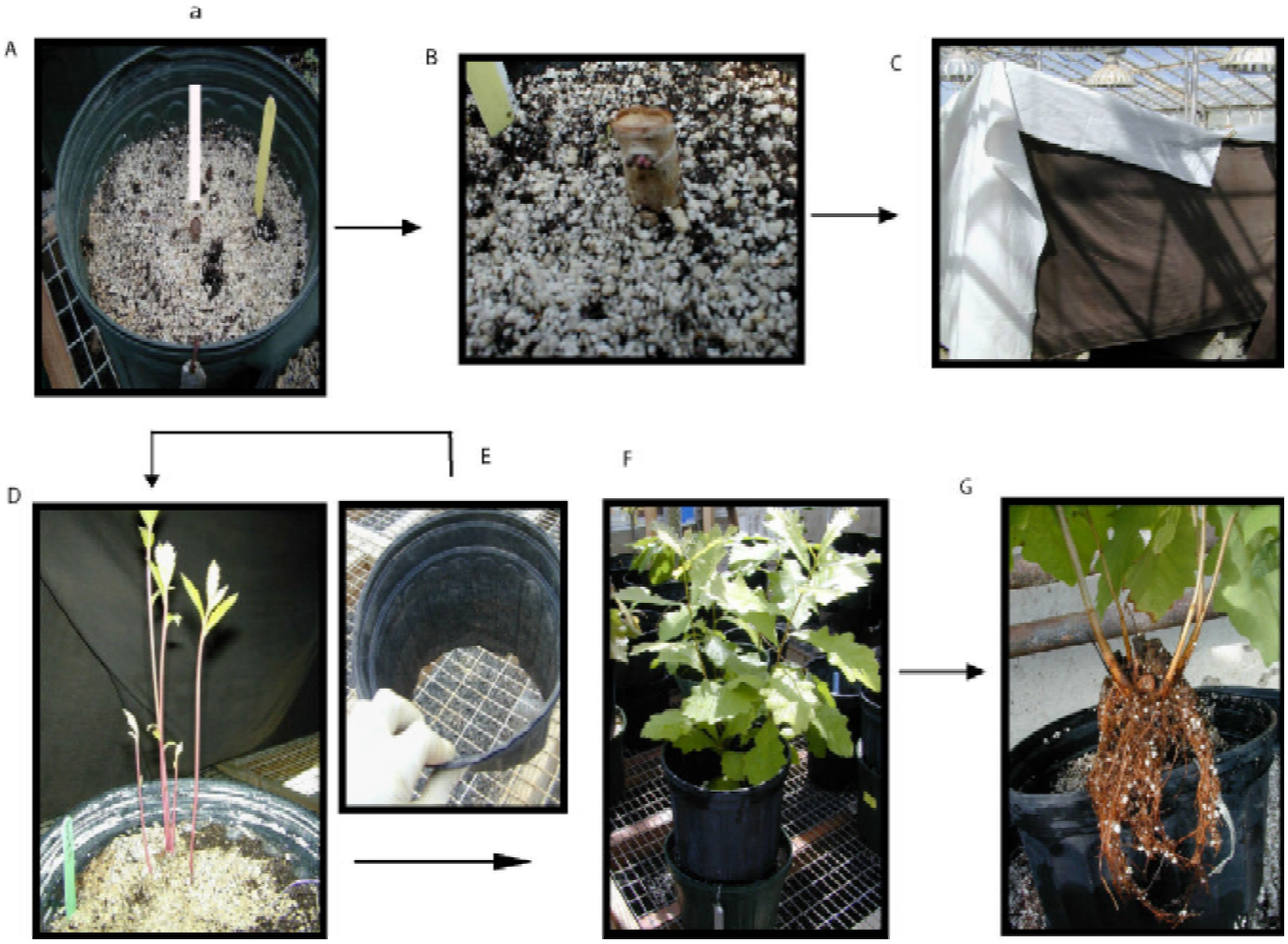

Fig. 1. Container Layering Method: A. stock plant cutback to soil level; a. arrow pointing to cutback stem; B. budbreak on stock plant stump; C. black tent used to etiolate shoots; D. etiolated shoots ready for treatment; E. bottomless pot placed over treated etiolated shoots in D; F. plants growing in full light on greenhouse benches; G. rooted shoots still attached to stock plant.

etiolated shoot (Fig. 1 D) was painted with 10,000 ppm IBA dissolved in a solvent (aqueous DMSO, aqueous ethanol, or aqueous acetone, depending on the experiment). After the IBA solution had dried, a bottomless pot (Fig. 1 E) was placed over the stock plant so it rested on the soil surface in the stock plant container. A lightweight soilless potting medium (peat:perlite, 1:2 by vol) was filled-in around the treated etiolated bases leaving the shoot tips exposed. The black cloth, at this point was gradually raised over a one-week period to acclimate the emerging shoots to light. Plants were then allowed to grow in full light (Fig. 1 F) on greenhouse benches. During this time, the potting medium around the treated shoots was kept moist, with more potting medium added as the shoots grew to ensure the treated section stayed covered. Six weeks after treatment, the bottomless pots and potting medium were removed (Fig. 1 G) and percentage rooting recorded on shoots that survived per pot. We conducted five experiments using this container layering technique, with variations in the methods for each experiment outlined in the next section.

Experiment 1: Gibberellin (GA_{4+7}) effect on budbreak and rooting of shoots in Q. bicolor. To test the effectiveness of Gibberellin (GA_{4+7}) in increasing the number of growing buds per stock plant stump (Fig. 1), an experiment was conducted using *Q. bicolor* in a completely randomized design with 16 plants per treatment. Three drops of Tween 20, a wetting agent, was added to 500 ml of 500 ppm GA_{4+7} and to 500 ml of water (control). Approximately 8 ml of each treatment solution was applied every 4th day until runoff, to the cutback stumps of each plant using a 3.8 cm wide paintbrush. We counted the number of swollen buds per stock plant stump for each unit before placing them beneath etiolation tents. Data were taken on the number of buds that broke per stock plant, and used to determine the cumulative number of buds per treatment category. The etiolated shoots were treated with 8,000 ppm IBA in 10% DMSO then layered as described using the container layering technique and data taken on percentage shoots that rooted per plant that survived.

Experiment 2: Gibberellin (GA_{4+7}) effect on budbreak and rooting of shoot in Q. macrocarpa. Based on results from experiment 1, a second experiment to investigate the effect of gibberellin (GA_{4+7}) application frequencies on budbreak was conducted, this time using 10 *Q. macrocarpa* plants per treatment (due to unavailability of *Q. bicolor*). In this experiment, three treatment levels were used: water (control), gibberellin (500 ppm of GA_{4+7}) applied every fourth day or every other day. Information was taken on the number of

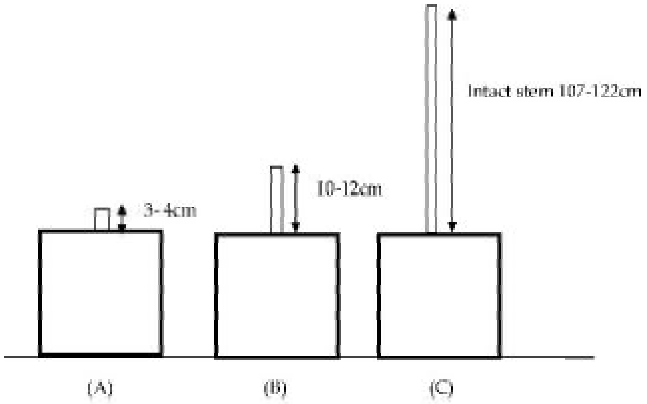

Fig. 2. Diagram of experimental set up showing degree of stock plant cutback.

buds that broke per stock plant, and used to determine the cumulative number of buds per treatment category. After counting the number of swollen buds per unit, stock plants were placed beneath black cloth tents for approximately 1–2 weeks until etiolated shoots had reached the desired height of 10–12 cm (~3.9–4.7 in). Etiolated shoots were then treated with 8,000 ppm IBA in 10% DMSO and layered as described using the container layering technique. Percentage rooting of shoots per pot was taken on plants that survived.

Experiment 3: Stock plant age on rooting. This experiment was designed to test the hypothesis that rooting decreased with increasing stock plant age. Stock plant age ranges of 2 yrs, 3–4 yrs, and 5–6 yrs of *Quercus bicolor* were used and 9–11 plants assigned within an age range. Using the container layering technique, etiolated shoots were treated with 10,000 ppm IBA dissolved in 10% DMSO. After taking data on rooting percentages, we cut back stock plants into old wood, smoothed the surface using a sanding machine, and confirmed the stock plants age by counting the number of annual rings.

Experiment 4: Effect of shoot origin on rooting. Quercus bicolor stock plants were dug from the field on December 7, 2001, and potted into #5, #7 and #15 pots. The plants received a 3-month chilling period at a temperature of 2.2C (36F). Nine to eleven plants were used per treatment. In the first treatment group, stock plants were cut back leaving a 3–4 cm (~0.2–1.6 in) stump above soil level (Fig. 2 A). In the second group a 10–12 cm (~3.9–4.7 in) stump height (Fig. 2 B) and in the third group (Fig. 2 C) plants were left intact (approximately 107–122 cm/~42.1–48.0 in) with buds allowed to break only at the distal portion of intact stems (buds that broke lower down the stock plant stem were rubbed off). Upon budbreak, plants were placed beneath black cloth tents and etiolated shoots treated with 8,000 ppm IBA dissolved in 10% DMSO and layered as described using the container layering technique.

Experiment 5: Effect of indole butyric acid solvents on rooting. Seven to twelve 3-year-old *Q. bicolor* stock plants were used per treatment in a completely randomized design. Upon budbreak, stock plants were placed beneath etiolation tents as described using the container layering technique. There were six treatments. The basal 4 cm portion of each etiolated shoot was treated with 10,000 ppm IBA dissolved in either 50% or 100% acetone; 10% or 5% DMSO; or 98% ethanol. The control treatments constituted the 'solvent only' treatments (with no IBA) of 10% DMSO, 100% acetone and 98% ethanol.

Rooting percentages were transformed using arc sin transformation = [arcsine (percentage rooting)$^{-1/2}$] and data subject to analysis of variance using the GLM procedure. Transformed rooting percentages were converted back into percentages before presented in the table of results. Means separation was accomplished using Tukey's (HSD) or Fisher's Protected LSD test with a level of significance at p = 0.5.

Results and Discussion

Gibberellin (GA_{4+7}) effect on budbreak and rooting of shoots. Gibberellin (GA_{4+7}) applied once every fourth day increased budbreak over a six-week period in *Q. bicolor* (Fig. 3). Gibberellic acid has been found to be effective in increasing budbreak in a number of plant species including citrus (16) and oaks (6). Based on these results a second experiment was set up to compare the effect of GA_{4+7} application frequencies on budbreak in *Q. macrocarpa*. Gibberellin (500 ppm GA_{4+7}) applied every 4th day produced more budbreak than the control or GA_{4+7} applied every other day. Mean number of buds per plant per week was twice as much in GA_{4+7} treatments than in the control (Fig. 4). Rooting results from both experiments 1 and 2 were similar in that GA_{4+7} applied to induce budbreak did not improve rooting (Table 1). Findings from our research show that gibberellin did not inhibit rooting in shoots, agreeing with Hansen (10) that the inhibitory effect of gibberellin is dependent on time of application, with the effect being strongest when applied during the early stages of root development (4, 9).

Effect of stock plant age on rooting. Stock plant age had no negative effect on the rooting of shoots from stock plants

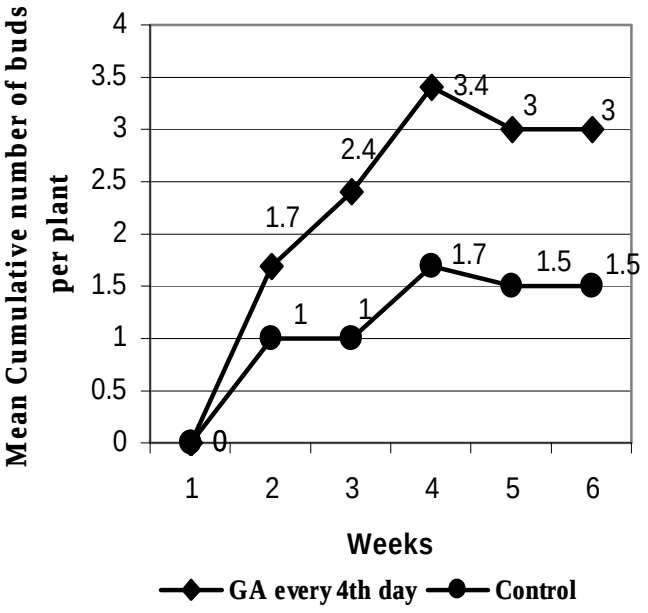

Fig. 3. Mean cumulative number of buds breaking in *Q. bicolor* per plant per week; number of buds that broke in GA treatment at the end of the 6-week period was significantly greater than in the control (at p = 0.05).

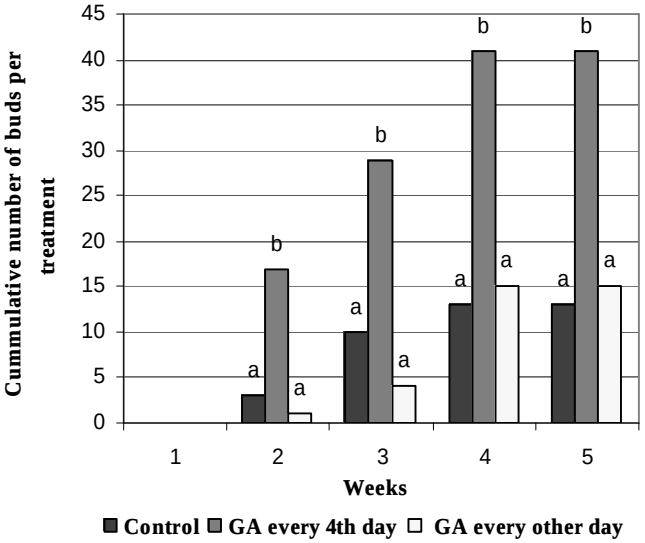

Fig. 4. Cumulative number of buds per treatment per week in *Q. macrocarpa*; treatments did not show significant differences at the end of the 5-week period at p = 0.05 but at p = 0.09.

2–6 years old, unlike other observations in cutting propagation, where rooting decreased with increasing age (12, 13). Rooting percentages of 77.8% (se =14.7), 63.6% (15.2) and 76.9% (11.4) were observed for the respective age ranges of 2 yrs, 3–4yrs and 5–6 yrs using 10,000 ppm IBA dissolved in 10% DMSO. We believe stock plant age had no effect on rooting because of the severe cut back of the plant's stem, which is recognized to be effective in rejuvenating and maintaining plants in a juvenile state (10).

Effect of shoot origin on rooting. Rooting was greater (Table 2) in stock plants cut back to a 3–4 cm stump above soil compared to shoots from intact 107–122 cm (~42.1–48 in) tall plants. Similar results were obtained in Douglas fir (*Pseudotsuga menziesii*), where cuttings taken from basal shoots had higher rooting abilities compared with cuttings taken from the upper more mature portions of the plant (1). A similar trend also reported by Roulund (15) in Norway spruce (*Picea abies).*

Effect of indole butyric acid solvents on rooting. Quercus bicolor shoots treated with 10,000 ppm IBA in 98% ethanol, 50% and 100% acetone rooted the highest, while shoots treated with IBA dissolved in 5% and 10% DMSO resulted in lower rooting percentages (Table 3). The number of roots per treated shoot increased with percentage rooting.

However, severe toxicity was observed in the form of shoot and leaf browning in apical regions of shoots treated with IBA dissolved in 5% and 10% DMSO (data not shown). These toxic effects were seen as early as 2 hours after treatment, progressing after 3–5 days into blackening of the apical 5–8 cm (~2.0–2.4 in) of the shoot with total shoot defoliation in that region. We found that these shoots could be saved most of the time by cutting off the damaged portion just above a node of healthy tissue. In agreement with experiments by Bonaminio and Blazich (2, 3) our experiments showed the importance of dissolving IBA in a suitable rooting solvent to allow easy uptake into plant tissue, as no roots were observed in shoots treated only with solvents.

Table 1. Effect of GA_{4+7} applications to stock plant on rooting in *Quercus bicolor* and *Quercus macrocarpa.*

Treatment	Rooting percentage (%)	No. of stock plants/treatment
Quercus bicolor		
Control[y]	46.9	16
GA applied every 4th day	65.6 NS[z]	16
Quercus macrocarpa		
Control[y]	33.3	6
GA applied every 4th day	50.0	7
GA applied every other day	38.1 NS[z]	7

[z]not significant at p = 0.05.

[y]water.

Table 2. Effect of shoot origin on rooting in *Quercus bicolor.*

Height (cm)	Rooting percentage (%)	No. of plants/treatment
3–4 cm	35.71a[x]	8
10–12 cm	14.75ab	8
107–122 cm (Intact)	1.82b	11

[x]Letters indicating means separated using Fischer's Protected LSD, at p = 0.05.

Table 3. Effect of 10,000 ppm IBA carriers on rooting and average number of roots per shoot in *Quercus bicolor*.

Treatments	Rooting percentage (%)	Average roots per rooted shoot	n[z]
10% DMSO only	0.0 (0.0)[y]c[x]	0.0b	9
IBA in 5% DMSO	28.6 (16.4)c	0.5b	7
IBA in 10% DMSO	65.0 (15.0)b	6.7a	10
100% Acetone only	0.0 (0.0)c	0.0b	9
IBA in 100% Acetone	100.0 (0.0)a	14.0a	8
IBA in 50% Acetone	81.3 (13.2)a	10.7a	8
98% Ethanol only	0.0 (0.0)c	0.0b	9
IBA in 98% Ethanol	84.3 (10.5)a	9.8 a	5

[z]Number of potted plants per treatment

[y]Standard error

[x]Letters indicating means separated using Fischer's Protected LSD at p = 0.05

Rooting percentages of oaks using this container layering technique were highest when 10,000 ppm of IBA was dissolved in less toxic auxin solvents like acetone and ethanol. Also, 500 ppm GA_{4+7} applied every fourth day was most effective in increasing the number of buds growing per cut back stock plant. Interestingly, unlike in cutting propagation, rooting percentages did not decline as the stock plant aged, at least for 6 years. Lastly, severe cutting back of the stock plant prior to layering improved rooting in shoots originating from the proximal parts of the stem. These results have moved us a step closer to achieving our ultimate goal of developing a reliable and commercially applicable technique for cloning oaks.

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