Improved Adventitious Rooting In *Quercus* Through The Use Of A Modified Stoolbed Technique

by George Hawver
and
Nina Bassuk
Department of Horticulture
Cornell University, Ithaca NY 14853

Vegetative propagation of the oaks (*Quercus*) is a difficult undertaking. Previous attempts at propagation using the methods of grafting, cutting propagation, and tissue culture have produced only very limited success (Drew and Dirr, 1989; Zaczek et. al., 1997). Recently, however, the propagation of oaks has been improved by using the practice of etiolation in conjunction with a modified stoolbed technique. A newer approach to improving propagation is through practices that are thought to reduce the plants' endogenous cytokinins, a class of hormones that is generally thought to inhibit adventitious rooting. The idea behind this approach is that a reduction in the plants' endogenous cytokinin levels may improve the potential for adventitious root formation. These two approaches to propagation, the use of etiolation in conjunction with the modified stoolbed technique and the use of practices aimed at reducing endogenous cytokinin levels, will be discussed here.

Etiolation

Etiolation, or the practice of excluding light from the plant environment, has previously been shown to improve adventitious rooting (Bassuk and Maynard, 1987). The benefit of etiolation on adventitious rooting comes, in part, by way of its positive influence on the shoots' anatomical development.

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Past research with *Carpinus betulus* indicated that by etiolating shoots during their initial development, the shoots would exhibit reduced lignification of the secondary xylem and reduced sclereid development. The net effect of these anatomical changes in the shoots seems to have been an increase in the potential sites for adventitious root development (Maynard and Bassuk, 1996).

The use of etiolation in conjunction with a modified stoolbed technique has also been used to improve adventitious rooting in *Quercus* (Griffin and Bassuk, 1996). This approach requires that the hormone auxin, mixed into an aqueous carrier (DMSO), be applied directly onto the plants' newly emerging shoots.

The Auxin/Cytokinin Relationship

When approaching work in vegetative propagation, one of the primary considerations is that of the relationship, and interaction, between the plant hormones auxin and cytokinin (Hartmann et al., 1990). Some researchers have suggested that these two hormones are the major endogenous factors in regulating adventitious root formation (Bollmark and Eliasson, 1990). The first of these hormones, auxin, has often been applied directly onto shoots (i.e., exogenously) to improve adventitious rooting. In some cases, auxin has been mixed into an aqueous carrier, which makes cell membranes more permeable to the auxin.

The second class of hormones that is thought to be an important endogenous factor in adventitious root formation, cytokinins, is produced in root tips and in seeds (Davies, 1995; Taiz and Zeiger, 1991). Typically, exogenous applications of cytokinins have inhibited root formation in cuttings (Van Staden and Harty, 1988; Okoro and Grace, 1978). It has been proposed that *endogenous* cytokinins will also inhibit adventitious root formation on intact plants (Nordstrom and Eliasson, 1991). A number of researchers have suggested that adventitious rooting would be improved by way of a reduction in endogenous cytokinin levels (Haissig and Davis, 1994; Blakesley, 1994).

Root Restriction

Researchers have speculated that root restriction, such as occurs when plants are maintained in small containers, inhibits the synthesis of cytokinins in the plant's roots (Dubik, et. al. 1989). Some of the common morphological responses to root restriction seem to be consistent with the suggestion that root restriction inhibits the production of cytokinins;

Table 1. Effect of etiolation on % stool shoot rooting.

Quercus species	Etiolation	White light
Q. macrocarpa	83 (se = 9.4)	52 (13.0)
Q. bicolor	64 (8.8)	29 (12.8)
Q. palustris	100 (0)	25 (17.1)
Q. accutissima	83 (9.0)	92 (7.2)
Q. imbricaria	36 (9.0)	10 (5.4)

Table 2. Effect of stock plant root restriction and root pruning on % stool shoot rooting under etiolated or white light conditions.

Quercus species	Etiolation	White light
Q. macrocarpa	Pot-bound 72 (se=1.1)	40 (8.7)
	Root pruned 37 (9.3)	52 (12.7)
Q. bicolor	Pot-bound 53 (6.8)	40 (9.6)
	Root pruned 24 (6.3)	35 (15)

these responses include reduced axillary shoot growth (Dubik, et. al., 1990; Robbins and Pharr, 1988; Tschaplinski and Blake, 1985, Richards and Rowe, 1977) and reduced leaf chlorophyll concentrations (Dubik, et. al., 1990; Tschaplinski and Blake, 1985; Hawver, 1997). In one root-restriction study, an analysis of cytokinin concentrations in the stem exudate of peach trees (*Prunus persica* L.) demonstrated a linear relationship and positive correlation between increasing container size and cytokinin concentrations (Alvarez, 1993).

Similarly, researchers in vegetative propagation who used a modified stoolbed technique have noted that adventitious rooting was promoted in avocado (*Persea americana*) when the rootstock plant was confined to a one quart container. However, when larger containers were used, propagation was less successful (Frolich and Platt, 1972; Frolich, 1966). Similar observations have been noted for propagation of *Castanea* (Caldwell, 1986).

Root Pruning

Because cytokinins are synthesized in root tips, and because the quantity of cytokinins that reaches the shoot is thought to reflect the extent of the plant's root system (Davies, 1995), it may be that by excising roots (tips), the plant's endogenous cytokinin levels will be temporarily reduced. Root pruning may, thus, be a practice that could be useful in stoolbed propagation.

End-of-Day Far Red Light

The ratio of red:far red light that plants receive at the end of their photoperiod is known to significantly influence growth and development. Application of end-of-day far red light (i.e., approximately 700-800 nanometer (nm) wavelengths) has produced plant responses that are noteworthy in that these responses have also been associated with low levels of endogenous cytokinins. Some of the common responses to end-of-day far red light include longer internodes on the main shoot, reduced axillary shoot growth, and reduced chlorophyll concentrations (Holmes and Smith, 1977; Kasperbauer, 1971). These particular responses suggest that an intermediary step in the plant response to end-of-day far red may be a reduction in endogenous cytokinin levels.

Based on what the literature suggested to us, we hypothesized that we would reduce the oaks' endogenous cytokinin levels and improve the potential for adventitious root formation by using end-of-day far red (EODFR) light, root restriction, and root pruning. We used these practices in conjunction with the modified stoolbed technique and, sometimes, with etiolation.

Materials and Methods:

Oak seedlings from five oak species (*Q. macrocarpa*, *Q. bicolor*, *Q. palustris*, *Q.*

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acutissima, and Q. imbricaria) were grown for 3 to 5 years in three to five gallon containers using a largely soilless medium 1:2:1 (soil: peat: perlite), by volume. In a (70 F day/ 60 F night) greenhouse just prior to bud break, we cut the shoots back so that only a 2 to 4 cm stub remained on the plant. After new buds were formed and began to swell, we placed the stock plants under black cloth tents and allowed the buds to grow out in near darkness (about 98% light exclusion) or allowed them to develop normally in full sunlight. It is important to prevent the shaded plants from overheating under the black cloth, so white plastic was draped over the black cloth while fans placed inside the tents served to keep the air temperature only a few degrees warmer than the temperature outside the tents.

The shoots under black cloth grew quickly and looked characteristically etiolated (no chlorophyll, long internodes, underdeveloped leaves). When shoots from either light or etiolated treatment grew to between 13 to 25 cm long, we painted their bases with a solution of 8000 PPM indole butyric acid (IBA) dissolved in 20% dimethyl sulfoxide (DMSO) in 50% ethanol, and 30% water, careful not to get the solution onto the growing tips. We then placed a bottomless pot, which was slightly smaller than the stock plant container, over the shoots allowing it to rest on the stock plant soil surface. After the IBA dried, a light, soilless mix of equal parts peat and perlite was added to the shoots contained within this 'chimney' pot. Moist media was added about half way up the stems, leaving the growing points exposed. The black cloth was gradually taken away over the period of one week to allow for acclimation of the etiolated shoots to the light. The shoots greened up quickly and began to look more like normal oaks. As the shoots grew, we added more media to the 'chimneys' so that at least 15 cm of stool shoot stem remained covered. The experiment ran for 4 months after which time the chimneys and media were removed and rooting assessed.

In some treatments, the root system was root-pruned while dormant and repotted so that approximately 5 cm of media separated the cut root tips from the container wall. All other plants were pot-bound in their containers, with large diameter roots pressing directly against the container wall. End-of-day far red lamps or incandescent lights, which are rich in far red, were

Table 3. Effect of end of day far red light (EODFR) supplements on % stool shoot rooting of stock plants grown only under white light.

Quercus species	White light only	White light plus EODFR	White light plus incandescent light
Q. macrocarpa	52 (se = 12.8)	64 (15.3)	72 (11.9)
Q. bicolor	29 (12.8)	24 (10.3)	43 (13.7)

applied to *Q. macrocarpa and Q. bicolor* under normal white-light conditions in the greenhouse. A 30 minute application of far red light or incandescent light was provided at 10:30 PM every night for the duration of the experiment. In order to disperse the heat that was produced by the lamps, box fans ran during the half hour that the lights were on. Fans ran for the control trees as well.

The far red light was achieved by filtering the light from 75 watt standard incandescent lights (General Electric) through a sheet of blue plastic (color #83) and red plastic (color #26) (Roscolux plastic, Rosco Laboratories, Inc. Stamford, Ct)). Twenty-four far red lamps were hung above a greenhouse bench that had dimensions of 8 x 6 feet (i.e., 48 square feet). The incandescent light was provided by hanging six soft white 60 watt incandescent light bulbs (General Electric) above a bench of the same dimensions. The incandescent bulbs and the far red lamps were hung approximately 1.5 feet above the plants. Above the bench that was for the incandescent light treatment and the bench for the controls, aluminum pie plates were hung to approximate the day-time shading that the trees underneath the end-of-day far red lamps received.

Results and Discussion:

1. *Test for Effect of Etiolation vs. No Etiolation on Percent Rooting*

For all species except *Q. acutissima*, the practice of etiolation improved adventitious rooting (Table 1). When the species are considered individually, the etiolation treatment produced greater percent rooting for *Q. macrocarpa, Q. bicolor, Q. palustris* and *Q. imbricaria*.. These results are consistent with previous reports of the positive effect of etiolation on rooting (Griffin and Bassuk, 1996; Bassuk and Maynard, 1987). These results confirm that when the modified stoolbed technique is used for oak propagation, etiolation should always be used. It may be, however, that etiolation should be used in conjunction with additional practices in order to realize an even greater improvement in adventitious rooting.

2. *Test for Effect of Root Restriction vs. Root Pruning, and Etiolation vs. White Light*

Results testing the effect of root restriction (i.e., use of pot-bound plants) versus root pruning in combination with etiolation or white light are shown in Table 2. This data suggests that percent rooting was generally greater in *Q. macrocarpa*, compared to *Q. bicolor*, and also that, in general, pot-bound plants exhibited a greater percent rooting than did the root-pruned plants. Although treatment effects are not necessarily statistically significant, the data for both species does suggest that the combination of root restriction and etiolation was the most effective means for improving adventitious rooting.

Although we did not quantify cytokinins in this study, it is possible that the root-restricted trees rooted better as a consequence of reduced endogenous cytokinins. The studies of other researchers have suggested that root restriction does reduce endogenous cytokinin levels (Alvarez, 1993).

Our root-pruning treatment seems to have been generally less effective than was root restriction. We had hypothesized that by excising roots, we could reduce the plants' production of cytokinins. It may be, though, that the process of excising roots, which was, effectively, a disturbance of the whole root system, produced more new white roots which in turn produced more cytokinin. Our results suggest that the use of root restriction does seem to be a valuable tool in propagation. Additional research into the effect of root restriction on adventitious rooting will be performed to gain certainty about the value of this practice.

3. *Test for Effect of End-of-Day Light Application on Percent Rooting*

The results for the end-of-day light application treatments on *Q. macrocarpa* and *Q. bicolor* are shown in Table 3. For *Q. macrocarpa*, the data suggests that a simple 30 minute ap-

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plication of EODFR or EODINC (i.e., far red-rich light) light may indeed improve adventitious rooting. Similarly for *Q. bicolor*, the results suggest that EODINC encouraged rooting. This data suggests that for both species, an application of EODINC may be more effective than an application of EODFR.

Evaluation of the output from our two light sources via an LI-1800 Portable Spectroradiometer (Li-Cor, Inc., Lincoln, Nebraska) indicated that the light quality provided by the treatments was as expected: the EODFR treatment supplied light of approximately 700 - 1100 nm; the EODINC treatment supplied light of approximately 400 - 1100 nm.

It should be noted, though, that while our EODINC and EODFR treatments differed in the quality of light that they provided, the treatments also differed in the *quantity* of light (micromoles /square meter/second) that they provided. Light quantity changes with changes in the orientation to and the distance from a light source. In our study, a single spectroradiometer reading was taken for each of the three light treatments; although meaningful data is not available regarding the differences in light quantity provided by our lamps, visual observation indicated that the EODINC lights provided much greater illumination than did the EODFR lamps.

Although we did not measure cytokinin concentrations, our results here are consistent with the idea that end-of-day light that is rich in far red would improve adventitious rooting, and that an intermediary step may include a reduction of endogenous cytokinins. While our study does not approach confirming our hypothesis, our results do indicate that additional research is warranted. It is important to note, however, that end-of-day light that is rich in far red, as we administered it to the stockplants, did not approximate the rooting advantage that was found through etiolation.

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