# Cloning the Old Main Oak (*Quercus macrocarpa* Michx.): Preliminary studies on stem cuttings and leaf explants.

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Figure 2. EIU campus, facing north, prior to 1912. Old Main is behind the grove of trees. Arrow indicates the Old Main Oak.

Figure 3. EIU campus, facing north, August 2012, shortly after significant pruning of the Old Main Oak.

**ABSTRACT -** Within the genus *Quercus* remains some of the most desirable tree species still without an efficient vegetative propagation protocol from mature tissue, including bur oak (*Q. macrocarpa)*. The objective of this study was to develop an effective disinfestation method to allow for cloning the Old Main Oak at Eastern Illinois University. The first step was to surface disinfest the starting material. Stem cuttings were treated with varying hormone levels and half were kept in darkness at 6C for 7d. The stems were sprayed with 0.087% chlorothalonil and dipped in 1.6%, 3.0%, 4.5% IBA or 0.1 µg/l TDZ before being placed in damp perlite (10/treatment). One series was placed under a 16 hr photoperiod and the other in darkness for 8 weeks. Stem cuttings treated with IBA did not respond as well as the TDZ-treated cuttings. The dark-treated cuttings reacted better than the cuttings exposed to light. Cold treated cuttings responded better. Some stem cuttings exhibited callus tissue, indicating cell division at the cut surfaces. Media for all leaf explants consisted of MS inorganics and 0.4 µg/l thidiazuron. Seven disinfestation treatments were examined, varying in $HgCl_2$ duration (30 sec to 40 min) and bleach concentration (10 or 20%) and duration (10 or 20 min). Each treatment was tested on 10 culture plates with 5 explants/plate. The proportion of tissue cultures showing contamination was 84%. The contamination decreased as bleach or $HgCl_2$ exposure times increased. A few of the cultures showed no fungal contamination and had exuded a halo of phenolic substances around the explants.

## INTRODUCTION

Also known as the bur oak or mossy cup oak, *Quercus macrocarpa* produces the largest acorns of any species and has a lifespan of around 400 years. Commercially, these oaks are some of the most valuable woods available due to its use in flooring, furniture, and cabinetry. Animals feed on the acorns and people enjoy their high shade area, making them desirable for non-commercial use, as well. Consisting of over 400 species, the *Quercus* genus is widely dispersed and can be found worldwide in temperate regions (Vengadesan, 2009). *Quercus* has a wide range of suitable environments in North America and can be found in hardiness zones 3 through 8 (Arbor Day Foundation, 2012; Figure 1).

Within the genus *Quercus* remains some of the most desirable tree species still without an efficient vegetative propagation protocol. To date, there have been no reports of successful micropropagation from mature tissue of this species, which is why we aim to develop a disinfestation and clonal propagation procedure.

Our tree of interest is the iconic Old Main Oak on Eastern Illinois University's campus. It predates the construction and founding of EIU and is estimated to be 250-300 years old (Meeker, 2012; Figure 2). It is a treasured landmark within the EIU campus and Charleston community as a whole, and it has been added to a register of historic landmarks by the Coles County Regional Planning Historic Preservation Advisory Council (Fopay, 2008).

Unfortunately, the tree was struck by lightning about ten years ago (Figure 3). Despite the tree's poor condition, there have been numerous confirmations that the tree is not suffering from disease, further exemplifying why this tree would be an exceptional specimen for asexual propagation (Meeker, 2012).

The objective of this study is to develop an effective disinfestation method to allow for the cloning of the Old Main Oak at EIU. The first step for cloning the tree with either tissue cultures or stem cuttings is to surface disinfest the starting material. Sterilization is important for the tissue culture treatment because any fungus or bacterium will thrive on the culture medium. However, limiting fungal growth on the stem cuttings is also important so that the cuttings can grow unimpeded from infection.

## MATERIALS & METHODS

**Vegetative Propagation from Stem Cuttings:**

Branches were collected (Figure 4) and terminal stems were trimmed to 7-15 cm with no leaves remaining. Half of the stem cuttings were immediately treated with varying hormone levels and the other half were kept in darkness at 6°C with their basal ends placed in deionized water for one week. The stems were sprayed with fungicide (0.087% chlorothalonil) and dipped in either 1.6%, 3.0%, 4.5% IBA or 0.1 µg/l TDZ before being placed in damp perlite. Fungicide but no hormone treatment served as the control. About 500 ml of perlite were placed in 1L beakers with 10 stem cuttings/ treatment (Figure 5). One series of hormone treatments were placed under fluorescent lights (16 hr photoperiod) and the other was placed in a cardboard box for light exclusion. About 100 ml $H_2O$ was maintained in each beaker to keep the perlite and stem cuttings moist. Eight weeks after the initial stem cuttings were made,all cuttings were rinsed and examined for root formation. Thus, the cold treated series was cultured for 7 weeks.

**Tissue Culture of Leaf Explants:**

Media for all explants consisted of MS inorganics and vitamins (Murashige & Skoog, 1962), 30 g/l sucrose, 100 mg/l inositol, 8 g/l agar (Sigma, Type A), and 0.4 µg/l TDZ. The culture medium was adjusted to pH 5.75, autoclaved at 15 p.s.i. for 20 min, and poured into sterile petri plates. Seven disinfestation treatments were examined starting with larger leaf sections (approximately 5 cm2) which were subsequently cut into explants before being placed on the media plates. Each disinfestation treatment was tested on 10 different culture plates with five explants (approximately 1.5 by 0.5 cm) per plate (Figure 5). The seven surface disinfestation treatments are shown in Table 1. The culture plates were kept in the culture room and checked after 4 and 7 days for fungal and/or bacterial contamination.

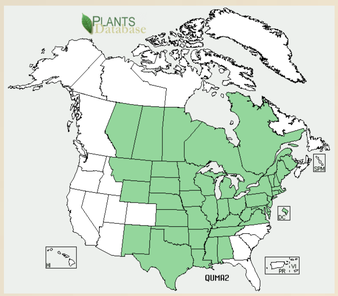


Figure 1. Distribution of *Quercus macrocarpa* from USDA PLANTS Database (http://plants.usda.gov).

Figure 4. Retrieving stem cuttings for vegetative propagation study, Sept. 6, 2012.

Figure 5. Stem cuttings in perlite.

Figure 6. Leaf explants in petri plates.

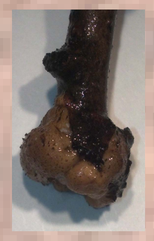

Figure 7. Callus growth from 0.1 mg/l TDZ, dark treatment.

Figure 8. Nodules of callus growth from cambial layer.

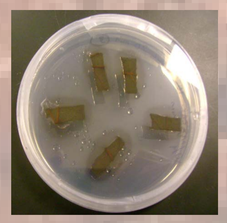

Figure 9. Halo of phenolics exuding from leaf explants.

Figure 10. Contamination originating from leaf explants.

Table 1. Influence of surface disinfestation treatment of leaf explants of *Quercus macrocarpa* on fungal infection rate after four days (10 plates/treatment; 5 explants/plate).

| Sodium hypochlorite | 70% Ethanol | 0.1% $HgCl_2$ | Contaminated explants/plate ± s.d. |
|---|---|---|---|
| 10%, 10 min. | 30 sec. | 30 sec | 5 ± 0.0 |
| 20%, 10 min. | 30 sec. | 30 sec | 5 ± 0.0 |
| 10%, 20 min. | 30 sec. | 30 sec. | 4.5 ± 1.6 |
| 20%, 10 min. | 30 sec. | 10 min. | 4.7 ± 0.5 |
| 20%, 10 min. | 30 sec. | 20 min. | 4.3 ± 0.7 |
| 20%, 10 min. | 30 sec. | 30 min. | 3.9 ± 1.6 |
| 20%, 10 min. | 30 sec. | 40 min. | 3.1 ± 1.9 |

## RESULTS

**Vegetative Propagation from Stem Cuttings**

Results from the light-excluded cuttings ranged from no changes, to signs of root induction. The 1.6% and 3.0% IBA-treated cuttings showed no indications of cell division or root induction and were in nearly in original condition, except for slight fungal growth below the perlite line. There was no fungal growth or cell division evident in the 4.5% IBA-treated cuttings. Five of the stems treated with 0.1 mg/l TDZ showed signs of cell division and callus growth. One TDZ-treated stem cutting showed possible root initiation at site of callus (Figure 7). The control showed signs of cell division with swelling in three cuttings. Cuttings maintained under a 16 hour photoperiod were less varied than the series maintained in darkness. One cutting that was exposed to 1.6% IBA had no nodules, but was slightly swelled. Cuttings exposed to 3.0% IBA, 4.5% IBA, and 0.1 mg/l TDZ were unchanged. The stem cuttings of the control were free from visible fungal infection, but showed no growth or root induction.

The light-excluded cuttings pretreated for a week at 6º C also showed a range of results. Two cuttings from the 1.6% IBA treatment had signs of cell division. One cutting had a single nodule of growth at the cut end and one had split the bark with cell growth above the basal end. For the 3.0% IBA-treated cuttings, one had nodules at axial locations that appeared on the verge of rupturing the bark. The 4.5% IBA treatment had 1 stem cutting that still showed green bark and a definite swelling at the base. Two other stem cuttings had nodule growth at axial positions. One of the cold treated, light excluded control stems had nodule growths from the cambial layer forming a ring (Figure 8).

For the cuttings pretreated at 6º C and exposed to a 16 hour photoperiod, one cutting treated with 1.6% IBA and two cuttings treated with 3.0% IBA had signs of cell division, as well as fungal contamination. There was no response by the 4.5% IBA treated stems. There were two stems displaying cell growth in the control.

**Tissue Culture of Leaf Explants**

Bleach concentration and exposure times were varied and additionally, mercuric chloride exposure times were increased with each treatment showing different results (Table 1). The overall proportion of cultures showing contamination was high (4.2 ±1.3). The rate of contamination, from the 4th day to the 7th day, decreased when bleach or mercuric chloride exposure times were increased. A few of the cultures (*e.g.*, 10% bleach, 20 min.) showed no fungal contamination and had exuded a halo of phenolic substances around the explants (Figure 9). A majority of the other cultures developed fungal, and possibly bacterial, contamination (Figure 10).

## DISCUSSION

The ultimate goal of this research is to clone the Old Main Oak on the EIU campus. To be successful, the plant material first needed to be disinfested. Some cuttings treated with a fungicide (0.087% chlorothalonil) still showed fungal contamination, while others that were not treated had no signs of fungal contamination. We are planning to examine additional fungicides for their efficacy. Stem cuttings treated with IBA did not respond as well as the TDZ-treated cuttings. One reason for this difference may be because TDZ is a cytokinin and encouraged cell division and also because it was applied as a liquid which may have penetrated more deeply than the powdered IBA. The fact that the cuttings reacted at all is encouraging because we know that the stems were still responsive, even though they were collected late in the season. The dark-treated cuttings reacted better than the cuttings exposed to light. Furthermore, cold treated stem cuttings responded better than the room temperature treated cuttings, possibly because the cold environment helped stabilize the cuttings, or that it decreased fungal growth. The swelling of basal ends, callus formation, and cracking of the bark from cell division is promising because if we can get those cells to divide, we may be able to stimulate some cells to initiate roots with modifications to the auxin treatment. In future experiments, cuttings will be placed in a misting chamber, where fungal growth will likely become less of an issue (Gocke *et al.,* 2008). The stem cuttings will also be treated with hormones in solution (rather than in powdered form) to increase their absorption.

The tissue cultures had mostly no results and were contaminated, but a few were axenic, which is encouraging. It was observed that the contamination all originated at the site of the explants, suggesting that it was not due to inadequate sterile technique. Microbial contamination, along with an accumulation of phenolics exuded into the medium, likely inhibited explant success. Tissue cultures without contamination will be moved to fresh media to eliminate the inhibitory effects of phenolics, due to previous successful reports (Vieitez *et al.,* 1994). Tissue culture disinfestation will also include an initial cleaning of explants with detergents. Although our results were only minimally encouraging, we did get some leaf explants free of contamination and some cell division in stem cuttings. Overall, these experiments were a good starting point toward cloning of the Old Main Oak.

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