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Cover illustration. Wendy Brockman (*Quercus palustris*).
Photos. p. 9: James MacEwen (Michael Heathcoat Amory); p. 10: Guy Sternberg (8th International Oak Society Conference participants); p. 11: Charles Snyers d'Attenhoven (*Quercus stellata*); p. 13: Béatrice Chassé (*Q.* ×*fernowii*).

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***Quercus palustris* (Béatrice Chassé).**

In-Vitro Tools for the Ex-situ Conservation of *Quercus* Species

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Quercus species are iconic both in natural and managed landscapes, and the ex-situ conservation of threatened oaks is increasingly important. Oak seeds cannot be banked using traditional methods – they require alternative approaches, such as embryo axis or shoot tip cryopreservation for ex-situ conservation. Both methods involve in vitro culture, and we report here on two aspects of oak in vitro culture. First, a study was undertaken to improve the recovery medium for embryos of *Q. palustris* Münchh. that were stored in LN 22 years ago. A Design of Experiments (DOE) approach was used, specifically, a quadratic response surface design, to determine the effects of NO_3^-, KH_2PO_4, BAP, IAA, MS vitamins and the proportion of NH_4^+:K^+ in 33 treatment combinations. Using fresh embryos, the results indicated that a medium lower in BAP and N, but with some NH_4^+ was most beneficial. Second, shoots of *Q. shumardii* Buckley taken at three different times, were initiated into culture. Much browning and very little growth was observed except from explants from the second collection, which are being grown further to provide material for shoot tip cryopreservation studies. These results support reports that phenolics and the explant stage can have a significant impact on in vitro growth of oak cultures. In addition, the use of DOE as a systematic approach for improving growth in *Quercus* has been demonstrated. Continued research in these areas should improve the application of ex-situ conservation methods to the conservation of endangered species of *Quercus*.

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In Vitro Tools for the Ex Situ Conservation of *Quercus*

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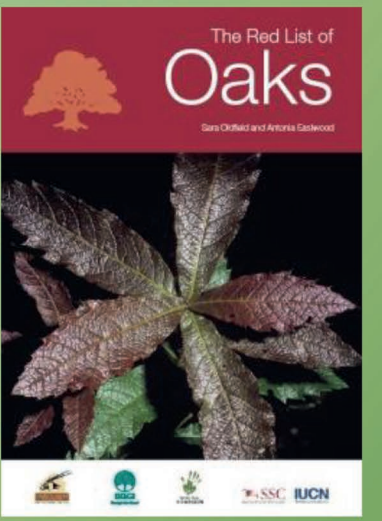
Introduction

- Oaks are an iconic and pivotal group of species, providing critical services to humans and to natural and developed landscapes worldwide.
- Of the 500 species of *Quercus,* only 175 have been sufficiently evaluated, but of these, 78, or 45%, have been identified as being of conservation concern
- Oak species are "exceptional species," in that the seeds cannot be seed-banked, because they cannot tolerate drying.
- Alternative methods, like embryo, bud, and tissue freezing , can be used for the conservation of exceptional species
- In vitro methods are important for these alternative approaches
- Overarching goal is to improve in vitro methods for oak conservation.
- Two studies are reported here that contribute to that goal.



Study 1

Goal: To improve recovery medium for isolated embryos of *Q. palustris* after cryopreservation.

Methods:
Excised embryonic axes of *Q. palustris* (Figure 1A) were sterilized and placed on 33 different treatments as determined by a Design of Experiments approach. Response surface methodology was used to test six factors that included NO_3 (10-40mM), PO_4 (1.2-3.6mM), BAP and IAA (0-1mg/L), MS

included NO_3 (10-40mM), PO_4 (1.2-3.6mM), BAP and IAA (0-1mg/L), MS vitamins (0-5X) and the proportion of NH4:K (0-0.5). Measured responses included quantitative measurements (presence, number, and length of roots, shoots, and leaves and presence of callus) and qualitative observations (color of the roots, quality of roots, shoots, and leaves, and overall look of the whole plant, Figure 1B).

Results:

•Overall, root growth benefitted from lower BAP and NO3, but a higher NH4:K ratio was beneficial (Figure 2).

•Higher BAP and NO3 levels inhibited leaf expansion (Figure 3) and negatively affected number of leaves as well as the overall appearance of the plants.

Conclusions: The improved medium for *Q. palustris* embryonic axes will contain a low amount of nitrogen and no plant hormones.

Next Steps: 1) The improved medium will be tested on cryopreserved embryonic axes; and 2) Embryonic axes stored for 22 years in liquid nitrogen will be removed from CREW's CryoBioBank.

Figure 1: A. Intact acorn of *Q. palustris* and cross-section, with embryonic axis indicated by red circle. **B.** Embryo with normal germination in vitro.

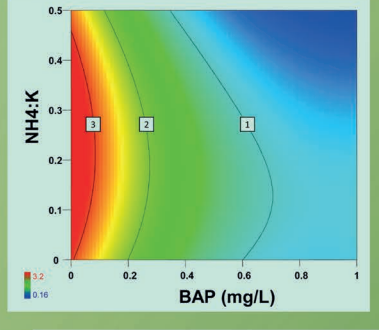


Figure 2. Effect of NH_4 proportion of and concentration of BAP on root growth.

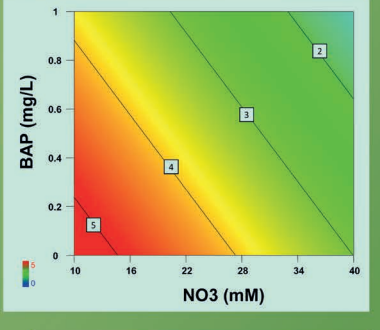


Figure 3. Effect of the concentration of BAP and NO3 on leaf expansion.

Red = strongest response, most roots (Fig 2); most leaf expansion (Fig 3).

Study 2

Goal: To improve the initiation of shoot propagating cultures from shoots from mature trees.

Methods:

Emerging shoots from trees of Q. shummardii were removed in the spring of 2015 at three stages: 1) swelling and beginning elongation; 2) beginning leaf expansion, and 3) young expanded leaf stages. Shoots were surface sterilized and cultured on Woody Plant Medium with 0.2 mg/L BAP and 100 mg/L benlate.

Results:

•Little or no growth was observed from shoots taken at stages 1 and 3, and the shoots became dark brown (Table 1; Figure __).

•Shoots taken at stage 2, while showing some browning, also showed the outgrowth of multiple lateral buds (Table 1; Figure __).

•These shoots could be subcultured, with continued, sporadic growth.

Conclusions: The developmental stage of the explant is an important component in the initiation of shoot cultures from *Q. shummardii*.

Next Steps: 1) A more detailed study of the developmental stages and their growth in culture of *Q. shummardii;* and 2) Evaluation of the effects of stage on the initiation of cultures from other species of *Quercus*.