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contact
Béatrice Chassé
pouyouleix.arboretum@gmail.com or editor@internationaloaksociety.org
Les Pouyouleix
24800 St.-Jory-de-Chalais
France

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Table of Contents

—/ 11 /—
Foreword
Twenty-one Years After
*Charles Snyers d’Attenhoven*

—/ 13 /—
Preface
From Small Acorns
*Sara Oldfield*

—/ 15 /—
Introduction
Oak Research in 2015: a Snapshot from the IOS Conference
*Andrew L. Hipp*

—/ 23 /—
Systematics and Biogeography of the American Oaks
*Paul S. Manos*

—/ 37 /—
Diversity, Distribution and Ecosystem Services of the North American Oaks
*Jeannine Cavender-Bares*

—/ 49 /—
Drought Tolerance and Climatic Distributions of the American Oaks
*Matthew Kaproth and Jeannine Cavender-Bares*

—/ 61 /—
Phylogeny and Introgression of California Scrub White Oaks (*Quercus* section *Quercus*)
*Victoria L. Sork, Erin Riordan, Paul F. Grugger, Sorell Fitz-Gibbon, Xinzeng Wei, and Joaquin Ortego*

—/ 75 /—
A Tough Little Survivor: The West Texas Oak, *Quercus hinckleyi*
*Janet Rizner Backs*

—/ 83 /—
Landscape and Conservation Genetics of the Island Oak, *Quercus tomentella*
*Mary V. Ashley, Janet R. Backs, and Saji T. Abraham*

—/ 91 /—
Hybridization and Adaptive Divergence in Oaks
*Olivier Gailing and Jennifer Riehl*
Asexual Propagation of Oak Hybrids: Our Progress, and the Challenges of Producing Clonal Plants
Nina L. Bassuk, Bryan R. Denig, and Miles Schwartz Sax

Eating Acorns: What Story do the Distant, Far, and Near Past Tell Us, and Why?
Béatrice Chassé

New and Lesser-Known Cultivars 2013-2015
Ryan Russell and Eike Jablonski

Anther Culture of Turkey Oak (Quercus cerris)
Joseph Rothleutner

The Plant Collections Network and the Quercus Multisite Collection
Greg Paige

Rescuing Plant Species with Extremely Small Populations in China: the Case of the Xichou oak, Quercus sichourensis
Weibang Sun, Zhekou Zhou, Wenyun Chen, Yuan Zhou, Lei Cai, Murphy Westwood, and Jessica Turner

Conservation of Quercus arbutifolia, a Rare Oak, from Southern China’s Montane Cloud Forests
Min Deng, Xu Jun, Yi-Gang Song, and Xiao-Long Jiang

A Genetic Map for the Lobatae
Arpita Konar, Olivia Choudury, Oliver Gailing, Mark V. Coggeshall, Margaret E. Staton, Scott Emrich, John E. Carlson, and Jeanne Romero-Severson

Development of New Genomic Resources for Northern Red Oak, Quercus rubra
Christopher R. Heim, Mark V. Coggeshall, Arpita Konar, and Jeanne Romero-Severson

Sustaining Oaks in the Chicago Region Landscape: Developing a Plan for Maintaining Oak Dominance in an Urban Landscape
Lindsay Darling and Robert T. Fahey

Pathfinder: the Last Prairie Sentinel
Guy Sternberg

Oaks in Puebla: Growing Successes and Failures, and New Research Topics
Maricela Rodríguez-Acosta, Allen J. Coombes, Carlos A. Paredes-Contreras, Stephanie Fernández-Velázquez, and Citlali Guevara-González

Searching for the Hardy Southern Live Oak
Anthony Aiello
<table>
<thead>
<tr>
<th>Page</th>
<th>Title</th>
<th>Authors/Contributors</th>
</tr>
</thead>
<tbody>
<tr>
<td>233</td>
<td>The Last Basketmaker: Indiana’s Forgotten History of Oak-Rod Baskets</td>
<td>Jon Kay</td>
</tr>
<tr>
<td>245</td>
<td>Are Resource Dynamics a Necessity for Oak Masting?</td>
<td>Ian Pearse</td>
</tr>
<tr>
<td>255</td>
<td>Preserving Oak (Quercus sp.) Germplasm to Promote Ex-Situ Conservation</td>
<td>Christina Walters, Lisa Hill, Jennifer Crane, Marcin Michalak, Xia Ke, Jeffrey Carstens, Kevin Conrad, Murphy Westwood, Alison Colwell, Joanna Clines, and Pawel Chmielarz</td>
</tr>
<tr>
<td>267</td>
<td>The Pace of Microevolution of European Oaks During Environmental Changes</td>
<td>Antoine Kremer</td>
</tr>
<tr>
<td>277</td>
<td>Launching the Global Oak Conservation Initiative at The Morton Arboretum</td>
<td>Lisa Kenny and Murphy Westwood</td>
</tr>
<tr>
<td>290</td>
<td>Workshops</td>
<td></td>
</tr>
<tr>
<td>305</td>
<td>Poster Sessions</td>
<td></td>
</tr>
<tr>
<td>343</td>
<td>Pre-Conference Tour</td>
<td>Roderick Cameron</td>
</tr>
<tr>
<td>364</td>
<td>The Morton Arboretum</td>
<td>Charles Snyers d’Attenhoven</td>
</tr>
<tr>
<td>375</td>
<td>Post-Conference Tour</td>
<td>James Hitz</td>
</tr>
<tr>
<td>390</td>
<td>International Oak Society Service Awards</td>
<td></td>
</tr>
<tr>
<td>392</td>
<td>First International Oak Society Silent Auction</td>
<td></td>
</tr>
</tbody>
</table>
Quercus palustris (Béatrice Chassé).
In-Vitro Tools for the Ex-situ Conservation of Quercus Species

Valerie C. Pence¹, Anne-Catherine Vanhove¹, and Randall Niedz²

¹. Center for Conservation and Research of Endangered Wildlife
Cincinnati Zoo and Botanical Garden
Cincinnati, OH 45220, USA

². USDA-ARS-US Horticultural Research Laboratory
Ft. Pierce, FL 34945-3030, USA

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 NO₃⁻, KH₂PO₄, BAP, IAA, MS vitamins and the proportion of NH₄⁺:K⁺ in 33 treatment combinations. Using fresh embryos, the results indicated that a medium lower in BAP and N, but with some NH₄⁺ 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.
In Vitro Tools for the Ex Situ Conservation of Quercus

Valerie C. Pence¹, Anne-Catherine Vanhove¹, and Randall Niedz²
¹Center for Conservation and Research of Endangered Wildlife, Cincinnati Zoo & Botanical Garden, 3400 Vine Street, Cincinnati, OH 45220; and ²USDA-ARS-US Horticultural research Laboratory, 2001 South Rock Road, Ft. Pierce, FL 34945-3030.

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 NO₃ (10-40mM), PO₄ (1.2-3.6mM), BAP and IAA (0-1mg/L), MS
included NO₃ (10-40mM), PO₄ (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.

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**Study 2**

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

**Methods:**
Emerging shoots from trees of *Q. shumardii* 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. shumardii*.

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