

# The Challenges of *Ex-Situ* Conservation for Threatened Oaks

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## Introduction

Numerous challenges face individuals and institutions working to conserve threatened oak species. These challenges range from a fundamental lack of information on which species are threatened (and resulting inability to prioritize conservation activities) to growing threats that are difficult to predict and mitigate. Other challenges include widespread hybridization that may or may not be natural and which blurs the lines of which species and individuals require conservation action. These challenges all impact the survival of plants in the wild, presenting a need to ensure that off-site (i.e., *ex-situ*) collections are created as a safety net against extinction for threatened species.

Unfortunately, attempting to build *ex-situ* collections of oaks presents yet another challenge, as oak seeds are not able to be banked for long-term conservation. Curation of living collections are the only form of *ex-situ* conservation currently being carried out for threatened oaks, but for most species these *ex-situ* collections do not provide a sufficient safety net against extinction. Other forms of *ex-situ* conservation, including in vitro propagation and cryopreservation, are exceptionally challenging for most oak species due to the presence of tannins. Together, these challenges present opportunities for individual and collaborative work to secure threatened species of oaks before they are lost. Here, we discuss the specific challenges faced by oaks in more detail, and present results of a recent project attempting to surmount these challenges, particularly with respect to building *ex-situ* collections with high conservation value for four threatened oaks native to the United States.

### *Conservation challenge 1: Inadequate data*

The Global Tree Specialist Group recently attempted to assess the conservation status of all known oak taxa using IUCN's internationally-accepted criteria (Red List version 3.1), and found that only 175 out of 500 taxa had enough information to be fully evaluated (Oldfield and Eastwood, 2007). Of these, 56 taxa (32%) were assigned a Critically Endangered, Endangered, or Vulnerable status, with their continued survival in the wild threatened by a range of factors including habitat



*In vitro* propagated shoot of *Quercus georgiana*.

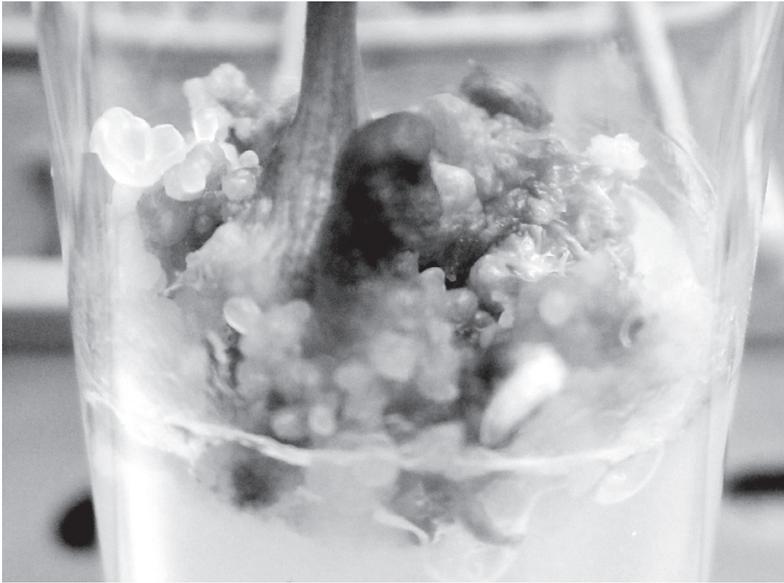
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destruction and degradation, invasive species, and unsustainable harvesting. The remaining c. 333 known taxa were either evaluated and found to be data deficient (33) or not evaluated (c. 300) because not enough information was available to begin the process. A clear conservation priority for these remaining 333 taxa is to gather enough data to allow full evaluation, which can then guide strategic conservation action.

#### *Conservation challenge 2: Unpredictable and accelerating threats*

For the 56 taxa known to be threatened with extinction in the wild, conservation action is needed to ensure plants are maintained in vigorous populations in their native habitat (i.e., *in situ* conservation) to support long-term survival and evolution. Unfortunately, the continued survival of oak species is increasingly at risk due to a combination of stresses relating to climate change and pathogens



Embryogenic culture of *Quercus arkansana*, with various stages of developing somatic embryos. photo©Valerie Pence

like Sudden Oak Death (*Phytophthora ramorum* S. Werres & A.W.A.M. deCock). Rapidly changing climates shift habitat and species distributions, so protected areas that support rare species *in situ* today may no longer be suitable for those species in the future (Hawkins, Sharrock, and Havens, 2008). And invasive pests and pathogens can unpredictably drive threatened as well as common species toward extinction (a great example comes from North American ash species and the introduction of Emerald Ash Borer in the United States). These stresses mean that *in situ* conservation and restoration must be accelerated to help species cope with multiple unpredictable threats, working to ensure populations are robust enough to allow species to migrate and/or adapt via evolution to avoid extinction. These stresses also mean that *in situ* conservation is a necessary but increasingly inadequate approach to ensure long-term conservation of oak diversity.

### *Conservation challenge 3: Hybridization*

Hybridization among oak species has been widely documented in both common and rare species. Hybridization has been shown to produce plants that successfully grow to adulthood and themselves reproduce (Penaloza-Ramirez *et al.*, 2010), but it may also lead to poor seedling performance that prevents plants from reaching adulthood (Curtu, Gailing, and Finkeldey, 2009). This poses challenges not only in identifying species and populations to protect, but also in determining appropriate management actions. Hybridization is listed as one of the threats to continued survival of a number of threatened oak species that are Red Listed as Critically Endangered (Oldfield and Eastwood, 2007).



Somatic embryo of *Quercus arkansana* with small but thickened cotyledons and radicle growth. photo©Valerie Pence

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#### *Conservation challenge 4: Recalcitrant seeds and high tannins*

To complement *in situ* efforts, the creation of genetically diverse, representative, and secure off-site (i.e., *ex-situ*) collections is needed to secure plant species diversity before it is lost. This is clearly spelled out in the Global Strategy for Plant Conservation (CBD, 2010), which sets the goal that 75% of the world's threatened species will be curated in secure *ex-situ* collections by 2020. For species with 'orthodox' seeds (able to be dried and stored at low temperatures for many years and still remain viable), *ex-situ* collections of stored seeds can

provide the greatest direct conservation value at the lowest cost. Unfortunately, seed banking is not an option for oaks because they have ‘recalcitrant’ seeds (not able to be dried and stored). Instead, *ex-situ* collection options for oaks and other ‘exceptional’ species that don’t produce bankable seed include *in vitro* or cryopreservation, as well as the curation of living collections. These collections must be well-designed and curated in order to provide high conservation value, and are more expensive to maintain than seed banks.

Currently, most *ex-situ* collections of oaks are held as living collections. Botanic Gardens Conservation International (BGCI) recently conducted a survey of threatened oak species in living collections (BGCI, 2009) that identified 3,796 oak records from 198 institutions in 39 countries. Fewer than half (26) of the 56 Red Listed oak taxa were identified among these collections. Further evaluation of records for these 26 taxa found in collections showed that the provenance for 82% of them was either unknown or of horticultural origin. This is a concern, as wild-collected material provides highest conservation value (*e.g.*, ability to be used to directly support restoration or reintroduction of the species), but only if it is well-documented and curated. In addition, many of the 26 taxa were only curated at one institution, sometimes as only one or a few plants. These collections may hold conservation value because they support research and education on the species, but they are not an adequate insurance policy against extinction. For this, the development of well-documented, wild-collected *ex-situ* collections that capture significant genetic diversity of the species and are replicated at numerous locations not susceptible to Sudden Oak Death are needed. Many living collections today do not meet these standards due to genetic issues such as having too little genetic diversity, of potential hybrid origin, being of unknown provenance, or being located in areas of high Sudden Oak Death threat.

Very few *ex-situ* collections of oaks are found in *in vitro* or cryopreserved collections today, largely because protocols have not been developed for most species. The high tannin content of oak tissues can pose challenges for *in vitro* work, but several species have been successfully propagated through shoot cultures or somatic embryogenesis (Pijut, Lawson, and Michler, 2011). Generally, seedling or juvenile tissues are most responsive, but *in vitro* propagation from mature tissues in a few species has been demonstrated (*e.g.* Vieitez *et al.*, 1994; Valladares *et al.*, 2006). Cryopreservation techniques have also been developed for a few species using zygotic embryo axes or somatic embryos (Gonzalez-Benito *et al.*, 2002; Pritchard, 2007; Fernandes *et al.*, 2008; Sanchez *et al.*, 2008). Freezing zygotic embryo axes relies upon using seeds, which are not always reliably produced by oak trees (*e.g.*, masting years and other potential delays in successful seed production due to disease outbreaks, natural or human-caused disasters, or other climatic stresses).

Additionally, the tendency of oaks to hybridize means that use of seed to develop *ex-situ* collections may be counterproductive, using resources to conserve plants *in vitro*, via cryopreservation, or in living collections that are hybrids which are not representative of the species and which may stand little chance of surviving in the wild (Curtu, Gailing, and Finkeldey, 2009). Cryopreserving zygotic embryo axes requires growth *in vitro* for post-thaw recovery, while freezing somatic embryos relies on first establishing *in vitro* cultures of these tissues. Thus, these

reports demonstrate both the need and the potential for utilizing in vitro methods for *ex-situ* conservation of oak. The critical conservation status of many oak species highlights the need for research to develop successful propagation and cryopreservation protocols from adult tissue and, following that, a need for work to build secure, genetically diverse and representative collections of the most threatened oak species.

A recent project on four threatened species in the United States demonstrates the type of work needed to advance *ex-situ* conservation of oak species. We outline progress on this project to-date, in the hope that the process and results will be helpful in developing more effective oak *ex-situ* collections in the coming years for all threatened oak species.

## Methods

### *Study Species*

Four oak species in the southeastern United States found to be threatened following the global Red List assessment (Oldfield and Eastwood, 2007) were selected for this project. This included the critically endangered *Quercus boyntonii* Beadle, the endangered *Quercus georgiana* M.A. Curtis and *Quercus acerifolia* (E.J. Palmer) Stoyonoff & Hess, and the vulnerable *Quercus arkansana* Sarg. (Table 1). Prior to this project, it was not clear how many living collections of these species were curated within the United States, or how secure and genetically diverse they are.

It is not known how susceptible these four species are to Sudden Oak Death (SOD). SOD has killed many oak trees in parts of California, and a strict quarantine process has been implemented to attempt to halt its spread. While these efforts have been mostly successful, SOD has been found in nurseries and detected in waterways in Georgia and Alabama (and many other states) and the distribution of these four oak species coincides with the areas of greatest risk for SOD in the US outside of the current outbreak in California (Kliejunas, 2010).

### Identifying living collections

To identify living collections for our four study species in the United States, we used BGCI's PlantSearch database. PlantSearch is the only global database of species maintained in the living collections of botanic gardens and other botanical institutions around the world (BGCI, 2011). We then contacted individual gardens that report cultivating each species in PlantSearch for additional detail about the number of plants maintained in their collections, and their provenance. These details were used to determine the potential conservation and research application of collections for each species, and to identify plants to support micropropagation research trials.

Following this data-collection, we asked gardens maintaining documented, wild-collected living collections of each study species to provide cuttings from their plants for use in micropropagation research trials. To our knowledge this was the first attempt to micropropagate any of these species.

Institutions providing cuttings were asked to collect at least 6 inches of new growth from each parent plant in the spring of 2011 following this protocol:

1. Take cuttings in early morning when the tree is well-watered to ensure shoots and leaves contain as much moisture as possible when they are shipped.
2. Identify one or more branches of new growth for a total of at least 6 inches of plant material per plant.
3. Use sterilized pruning shears to cut the stem of selected branches about 2 inches below the new growth at a 45 degree angle.
4. Place cuttings directly into plastic bags with moist paper towels. Cuttings from one tree can go together into one bag; place cuttings from different trees in separate bags.
5. If possible, repeat steps 2-4 for old growth material. Old and new growth cuttings can be placed in the same bag for each tree.
6. Seal plastic bag and write identifying information using permanent marking pen.
7. Ship material overnight to the Center for Conservation and Research of Endangered Wildlife (CREW) at the Cincinnati Zoo and Botanical Garden.

In total, cuttings from ten plants for each species were collected and sent to CREW for micropropagation research trials.

### **Micropropagation research trials**

Upon arrival at CREW, samples were evaluated for the developmental stage of the buds/leaves. Twigs with emerged leaves were immediately surface-sterilized in a 1:10 dilution of commercial bleach (diluted concentration approximately 0.5% chlorine) with 0.05% Tween 20 (a surfactant) for 10-15 minutes with stirring, followed by rinsing with sterile, purified water. Following sterilization they were placed into culture following protocols below. Twigs without emerged leaves were trimmed at the base, placed into water purified by reverse osmosis and maintained on the lab bench under ambient light and temperature. They were checked daily and when buds began to leaf out, buds and young leaves were removed, surface-sterilized as above, and placed into culture following protocols below. In a few cases, buds did not leaf out and these collections were not cultured.

To establish micropropagation trials, all media were gelled with 0.25% Gelzan (Caisson Laboratories, Inc.), unless agar is indicated in the protocols below, in which case a concentration of 0.8% agar (Sigma Chemical Co.) was used. For this research, two approaches were used: 1) *Bud culture* using young buds left intact on short pieces of stem to stimulate axillary shoot growth; and 2) *Leaf culture* using young leaves excised from buds to stimulate somatic embryogenesis.

*Bud culture.* Buds were cultured on six media: two concentrations of benzylaminopurine (BAP) – 0.89 and 4.44  $\mu\text{M}$  with three salt formulations: 1) Gresshoff and Doy (GD) (1972); 2) Woody Plant (WP) (Lloyd and McCown, 1980); and 3) Murashige and Skoog (MS) (1962), all containing 3% sucrose, Gamborg's organics (Gamborg, Miller, and Ojima, 1968), and 100 mg/L of the fungicide, benlate (benomyl; methyl 1-butylcarbomoyl)-2benzimidazolecarbamate; Sigma Chemical Co.). Culture tubes (25 mm x 150 mm) were used with translucent polypropylene closures, approximately 15 ml of medium/tube, with one bud per tube. One drop, approximately 0.05 ml, of a solution of filter sterilized antibiotics (5 mg/ml cefotaxime and 0.25 mg/ml vancomycin) was added to each tube

before incubating. Cultures were incubated at 26°C, with 20-30  $\mu\text{mol}/\text{m}^2/\text{sec}$  photosynthetically active radiation (PAR) under CoolWhite fluorescent lights, 16:8 hr light:dark photoperiod. Buds were transferred every 3-4 weeks onto the same media (lacking benlate and antibiotics) until the fourth transfer, when all were transferred to WP medium with 0.89  $\mu\text{M}$  BAP and agar, and subcultured on that medium thereafter.

**Leaf culture.** Young leaves were cultured using three procedures reported in the literature for somatic embryogenesis in other species of *Quercus* (described below). All leaves were cultured in 60 x 15 mm disposable petri plates, with approximately 15 ml of medium/plate and 3-6 leaves per plate. Leaves were cultured whole and the initial length of each leaf was measured, with sizes ranging from 0.3 to 1.2 cm. Leaf cultures were incubated in the conditions described for buds, except that in some cases, as indicated, leaf cultures were incubated in the dark. Benlate and antibiotics were added, as for bud cultures, to the first medium of all three procedures and omitted thereafter.

**Procedure 1.** Using a modification of the procedure of Hernandez *et al.* (2003) for *Q. suber* L. leaf pieces were cultured on MS medium with half-strength macronutrients plus 1% sucrose, 100 mg/L benlate, and 0.25% Gelzan for 7-14 days in the dark at 26°C, and then transferred to a medium with Schenk and Hildebrandt (1972) macronutrients, MS micronutrients, 3% sucrose and 0.25% Gelzan with 10  $\mu\text{M}$  BAP and 50  $\mu\text{M}$  naphthaleneacetic acid (NAA) for 30 days in the dark. Although leaves expanded on these media, very little callus and no somatic embryos were observed from these tissues, and they were not cultured further.

**Procedure 2.** Using a modification of the procedure of Toribio (2004) for *Q. robur* L., young leaves were cultured on MS medium with 500 mg/L casein hydrolysate (CH), 3% sucrose, 100 mg/L benlate, 0.25% Gelzan, plus 2.5  $\mu\text{M}$  BAP and 20  $\mu\text{M}$  NAA in the dark for 42 days. They were then transferred to fresh medium without benlate and with growth regulator levels reduced to 0.5  $\mu\text{M}$  BAP and 0.5  $\mu\text{M}$  NAA and maintained in light, as for buds, for 30 days. Embryos and embryogenic cultures were then transferred to MS medium plus 500 mg/L CH, 3% sucrose, 0.25% Gelzan, and no growth regulators, in culture tubes, as for buds.

**Procedure 3.** Using a modification of the procedure of Pinto *et al.* (2002) for *Q. suber* L. leaves were cultured on MS medium with 3% sucrose, 100 mg/L benlate, 0.25% Gelzan, plus 4.5  $\mu\text{M}$  2,4-dichlorophenoxyacetic acid (2,4-D) and 9  $\mu\text{M}$  zeatin riboside (ZR) for 3 weeks in the dark. They were then transferred to the same medium except with the growth regulators decreased to 0.45  $\mu\text{M}$  2,4-D and 2.25  $\mu\text{M}$  ZR and cultured in the light for 8 weeks. Embryos and embryogenic cultures were then transferred to culture tubes with MS medium plus 3% sucrose and no growth regulators. In addition to leaves, immature staminate and pistillate flowers were cultured with this procedure when they were available.

## Results

**Living Collections.** We identified a baseline of 18 *Quercus acerifolia*, 9 *Q. arkansana*, 22 *Q. boyntonii*, and 20 *Q. georgiana* plants in curated living collections in the United States. These are not sufficiently genetically diverse, secure *ex-situ* conservation collections for any of these species. However, the

species that had the strongest collection for conservation was the most threatened species; *Q. boyntonii*. This is because it had the greatest number of wild-collected plants with full provenance known (91% of the 22 plants identified, curated at 8 gardens). The species with the weakest collections for conservation was *Quercus arkansana*, which had only 10 identified plants in living collections at 5 gardens. Of these, only one plant was wild-collected and of fully known provenance. This limits the research and conservation application of *Q. arkansana* collections, and for this species, collections of unknown or horticultural provenance had to be used for the tissue culture trials.

**Bud Cultures.** Table 3 summarizes the results from the bud cultures that were initiated from these *Quercus* species. Among the four species, 0 to 9.5% of uncontaminated buds initiated shoot propagating cultures, with an overall rate of 1.4% (5 of 350 buds initiating shoot cultures). However, of these 5 buds, 3 were initially grown on WP medium with 0.89  $\mu\text{M}$  BAP and one other on GD medium with 0.89  $\mu\text{M}$  BAP. In addition, half of the remaining buds that were alive after 6 months but not producing shoots, were initially cultured on this medium. Thus, of the 94 buds that were cultured on WP medium with 0.89  $\mu\text{M}$  BAP, the culture initiation rate was 3%, and this medium was used for all later transfers and maintenance of these shoots.

Of the 569 buds (i.e., explants) cultured, there was an overall contamination rate (predominantly fungal) of 38.5%, but rates ranged from about 9 to 84 % depending on the source material (Table 3). *Quercus acerifolia* explants showed the highest rates of contamination, but of the 12 genotypes cultured of this species, contamination rates were highly variable, ranging from 0 - 100%. Shoot propagating cultures were successfully initiated from three of the four species tested (Figure 1).

**Leaf Cultures.** Table 4 summarizes the results from culturing immature leaves from the four *Quercus* species on three media protocols, directed at stimulating somatic embryogenesis. With Procedure 1, leaf tissue enlarged more than with the other two protocols, and this enlargement occurred on the first medium lacking growth regulators. When tissues were moved to medium with growth regulators, some pieces produced callus, although somatic embryos were not seen using this protocol. Most explants in Procedure 2 produced callus, and by 5-6 weeks, embryos were observed in one line of *Q. georgiana* and what appeared to be pro-embryos in lines of the other three species. After transfer to medium with reduced hormones, embryos became more apparent, and embryos from *Q. boyntonii* and *Q. georgiana* were moved to medium with no hormones for further growth (Figure 2). Leaf tissues in Procedure 3 also developed somatic embryos by 5-6 weeks, with embryos from *Q. arkansana* and *Q. georgiana* developing further. In both Procedures 2 and 3, some embryos enlarged on media lacking growth regulators and germination of the radicle was observed in some cases. In both protocols, some cotyledons became swollen and yellowish-orange in color, suggesting accumulation of pigments characteristic of acorn development. Overall, while cultured leaves in these experiments ranged from 0.3 - 1.2 cm in length, those that developed somatic embryos ranged from 0.3 - 0.8 cm in length. Somatic embryos also developed from some tissues of immature staminate flowers using Procedure 3 (data not shown).

## Discussion

Exceptional species such as oaks that do not produce storable seeds pose significant challenges for developing *ex-situ* collections, which provide a true safety net against extinction. It is not clear how many species fall into this ‘exceptional’ category, but it certainly includes species like oaks with recalcitrant seeds, as well as species that do not reliably produce viable seeds. Work to achieve the Global Strategy for Plant Conservation and ensure 75% of the world’s threatened plant species are held in secure *ex-situ* collections (CBD, 2010) must take exceptional species into consideration and develop methods to develop effective *ex-situ* collections. For species like oaks, it will be necessary to not only more effectively coordinate and curate living collections for conservation, but also to develop effective methods for micropropagation and cryopreservation.

In using four threatened oak species as a model for developing *ex-situ* collections for exceptional species, we see that there is potential to grow the conservation and research value of living collections in the United States by focusing on building more robust, collaborative collections that are wild-collected, of known provenance, and genetically diverse (*e.g.*, multiple individuals from multiple wild populations grown in multiple locations). Current living collections can provide some conservation and research value as-is (for example, we identified 20 of 22 identified *Q. boyntonii* trees at 8 institutions from known wild-collected sources). However, this is not the case for all four species; only 1 of 10 identified trees of *Q. arkansana* at 5 institutions was known from a wild-collected source. For all four of these species, and no doubt most ‘exceptional’ species currently found in living collections, there is a need and an opportunity to develop collections that provide much more conservation value with the same resources by phasing trees in cultivation from unknown or horticultural provenance to fully known, wild-collected material.

As the conservation value of individual collections is grown, it will be important to ensure these collections and expertise are connected, available to conservation practitioners and researchers, and ultimately able to be used to support research and conservation application. While it is not known exactly how many plants are needed in living collections to effectively capture genetic diversity in wild populations, a graduate student in the Longwood Graduate Program at the University of Delaware is currently working on one aspect of this for *Q. georgiana*, and similar research has been conducted on other exceptional species like palms (Namoff *et al.*, 2010).

Our results ultimately illustrate the importance of expanding our *ex-situ* conservation options for oaks, particularly for micropropagation and ultimately cryopreservation. Without this it will be difficult and cost-prohibitive to capture and conserve the genetic diversity needed to support potential reintroduction efforts should anything happen to wild populations. Fortunately, these results demonstrate that micropropagation procedures can be developed for at least three of the four species targeted in this study. *Q. arkansana*, *Q. boyntonii*, and *Q. georgiana* all show potential for both shoot micropropagation and somatic embryogenesis. *Q. acerifolia* did not respond, but also was more developmentally advanced than material of the other species when it was cultured. Leaves of *Q. acerifolia* were expanded to 2-3 cm in length, whereas, with the other three

species, only leaves 0.8 cm and smaller were responsive in the somatic embryo procedures. It is possible that younger tissues of *Q. acerifolia* might be more responsive, and this should be examined in the future.

These results have also identified procedures that should be useful in optimizing methods for these species in the future. One of six media tested for bud culture and two procedures for somatic embryogenesis gave the best responses and could be modified in the future to improve results. Although propagating cultures were established for three of these species, the rates of establishment were low and not every genotype responded. Reports of work with other *Quercus* species have also indicated low rates of culture initiation, particularly with material taken from mature trees (Vieitez *et al.*, 1994). However, our studies have suggested the importance of the developmental stage of explant tissue and have highlighted media that appear to be more effective than others in stimulating *in vitro* growth. Further work with these species can focus on optimizing those two factors, with the likely increase in the number of responsive genotypes and species.

In order to fully implement micropropagation and cryopreservation as tools for the *ex-situ* conservation of these species, procedures for rooting *in vitro*-propagated shoots, for converting embryos to plantlets *in vitro*, and acclimatizing plants to soil will need to be developed. Methods reported for other *Quercus* species can be used to guide this work (Martinez *et al.*, 2008; Vengadesan and Pijut, 2009; Vieitez *et al.*, 2009; Pintos, Manzanera, and Bueno, 2010). For long-term germplasm storage, cryopreservation in *Quercus* has been reported for embryogenic cultures using vitrification methods (Martinez, Ballester, and Vieitez, 2003; Valladares *et al.*, 2004). Apices from *in vitro* shoots have been successfully cryopreserved in other tree species (e.g. *Betula pendula*, *Robinia pseudoacacia*) using slow cooling and vitrification methods, suggesting their potential for *Quercus* species (Ryynanen, 1996; Verleysen *et al.*, 2005). With a coordinated utilization of these tools, multiple genotypes could be stored long-term as a resource for *Quercus* conservation in the future. As a group of species for which traditional seed banking is not an option, *Quercus* can serve as a model for the use *in vitro* and cryogenic techniques as alternative methods for *ex-situ* conservation.



*Quercus boyntonii* at the Hinds Road Glade, Gadsden, Alabama.  
photo©Guy Sternberg

**Table 1.** Information on study species, status in the wild, distribution, conservation ranks, and habitat descriptions.

| <b>Species name</b>       | <b>Common name</b>      | <b>Status in wild</b>   | <b>States</b>   | <b>Nature-Serve</b> | <b>RedList</b>        | <b>Habitat</b>  |
|---------------------------|-------------------------|---|---|---------------------|-----------------------|---|
| <i>Quercus acerifolia</i> | Mapleleaf Oak           | 6 populations, only a few 100's of individuals each.  | Arkansas  | G1                  | Endangered            | Open woods, ledges and cliff edges, and the rocky edges of plateaus.  |
| <i>Quercus arkansana</i>  | Arkansas Oak            | Over 200 (most healthy populations are in FL).  | Alabama, Arkansas, Florida, Georgia, Louisiana, Texas | G3                  | Vulnerable            | Sandy or sandy clay uplands or upper ravine slopes near heads of streams in deciduous woods.                            |
| <i>Quercus boyntonii</i>  | Boynton's Sand Post Oak | Only few populations in one county in Alabama, likely extirpated in Texas. Once considered extinct. | Alabama, Texas  | G1                  | Critically Endangered | Shrub layer of pine-oak forests on deep sandy soils in creek bottoms. Possibly also shallower soils of upland prairies. |
| <i>Quercus georgiana</i>  | Georgia Oak             | Scattered in Alabama, more populations in 14 counties in Georgia.                                   | Alabama, Georgia                                      | G3                  | Endangered            | Granite outcrops; dry slopes over granite   |

**Table 2.** Number of institutions in the United States holding living collections of the four study species identified using the GardenSearch database: includes number of identified plants that are wild collected with full provenance known, wild collected with full provenance unknown, as well as identified plants of unknown or horticultural origin are shown.

| Species              | No. institutions | No. wild collected plants |                         | No. plants unknown or horticultural | TOTAL PLANTS IDENTIFIED |
|----------------------|------------------|---------------------------|-------------------------|-------------------------------------|-------------------------|
|                      |                  | full provenance known     | full provenance unknown |                                     |                         |
| <i>Q. acerifolia</i> | 5                | 10 (56%)                  | 1 (6%)                  | 7 (39%)                             | 18                      |
| <i>Q. arkansana</i>  | 5                | 1 (10%)                   | 6 (60%)                 | 3 (30%)                             | 10                      |
| <i>Q. boyntonii</i>  | 8                | 20 (91%)                  | 2 (9%)                  | 0                                   | 22                      |
| <i>Q. georgiana</i>  | 12               | 4 (20%)                   | 15 (75%)                | 1 (5%)                              | 20                      |

**Table 3.** Contamination and number of shoot propagating cultures initiated from buds of four *Quercus* species after 7 months in vitro

| Species              | Total genotypes cultured | No. of buds | % contaminated | No. of clean buds | No. of shoot cultures initiated | % all buds producing shoot cultures | % clean buds producing shoot cultures |
|----------------------|--------------------------|-------------|----------------|-------------------|---------------------------------|-------------------------------------|---------------------------------------|
| <i>Q. georgiana</i>  | 8                        | 192         | 8.9            | 175               | 2                               | 1.0                                 | 1.1                                   |
| <i>Q. arkansana</i>  | 9                        | 163         | 24.5           | 123               | 1                               | 0.6                                 | 0.8                                   |
| <i>Q. boyntonii</i>  | 7                        | 25          | 16.0           | 21                | 2                               | 8.0                                 | 9.5                                   |
| <i>Q. acerifolia</i> | 12                       | 189         | 83.6           | 31                | 0                               | 0                                   | 0                                     |
| <b>Total</b>         | <b>36</b>                | <b>569</b>  | <b>38.5</b>    | <b>350</b>        | <b>5</b>                        | <b>0.9</b>                          | <b>1.4</b>                            |

**Table 4.** Somatic embryogenesis (SE) from young leaves cultured on three different media sequences, given as number and percent (in parentheses) of lines initiating SE cultures.

| Species              | Total genotypes cultured | <u>Procedure 1</u>     |                                      |                        | <u>Procedure 2</u>                   |                        |                                      | <u>Procedure 3</u>     |                                      |                                   | <u>Totals</u>                              |  |
|----------------------|--------------------------|------------------------|--------------------------------------|------------------------|--------------------------------------|------------------------|--------------------------------------|------------------------|--------------------------------------|-----------------------------------|--|--|
|                      |                          | No. of Leaves Cultured | No. (%) of lines initiating SE lines | No. of Leaves Cultured | No. (%) of lines initiating SE lines | No. of Leaves Cultured | No. (%) of lines initiating SE lines | No. of Leaves Cultured | No. (%) of lines initiating SE lines | Total leaves cultured per species | Total no. (%) of lines initiating SE lines |  |
| <i>Q. georgiana</i>  | 2                        | 37                     | 0 (0)                                | 36                     | 2 (6)                                | 33                     | 3 (9)                                | 106                    | 5 (5)                                |                                   |  |  |
| <i>Q. arkansana</i>  | 7                        | 66                     | 0 (0)                                | 64                     | 0 (0)                                | 68                     | 1 (1)                                | 198                    | 1 (0.5)                              |                                   |  |  |
| <i>Q. boyntonii</i>  | 4                        | 18                     | 0 (0)                                | 16                     | 1 (6)                                | 17                     | 0 (0)                                | 51                     | 1 (2)                                |                                   |  |  |
| <i>Q. acerifolia</i> | 11                       | 34                     | 0 (0)                                | 37                     | 0 (0)                                | 33                     | 0 (0)                                | 104                    | 0 (0)                                |                                   |  |  |
| <b>Totals</b>        | <b>24</b>                | <b>155</b>             | <b>0 (0)</b>                         | <b>153</b>             | <b>3 (2)</b>                         | <b>151</b>             | <b>4 (3)</b>                         | <b>459</b>             | <b>7 (2)</b>                         |                                   |  |  |

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*Quercus acerifolia* at Brown Spring, Magazine Mountain, Arkansas.  
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