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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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Bud-Forcing and Tissue Culture Propagation of *Quercus*

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Oaks (*Quercus* L.) are considered "recalcitrant" since they do not tolerate seed banking, and consequently, threatened species require alternative methods of protection. One method is tissue culture using newly flushed shoot tips. Two experiments were conducted to determine 1) the effectiveness of 6-benzylaminopurine (BAP), a cytokinin, on bud-forcing of *Quercus* and 2) the responses of species from the three North American *Quercus* sections (*Lobatae*, *Quercus*, and *Protobalanus*) across two different media in micropropagation.

Natural shoot emergence in the spring is in a narrow and somewhat unpredictable time window, but forcing bud break of cuttings can increase this window in a controlled environment. Experiment 1 involved dormant cuttings collected from 12 *Quercus* species placed into flasks of distilled water. Flasks were placed in a greenhouse with weekly BAP treatments: either 0, 100, or 500 ppm. Results indicate that BAP treatment at 100 or 500 ppm significantly increased the rate of bud break and shoot elongation by 1-2 weeks for four of the *Quercus* species, with no significant effect from BAP application on the remaining eight species. All except three *Quercus* species reached the target stage for micropropagation with all treatments, indicating that forcing bud break without BAP application is a viable option, but the rate in some species may be enhanced by BAP application.

In Experiment 2, newly flushed shoots were collected in the spring from 12 species of *Quercus* (different from those used in Experiment 1). Shoots were surface-sterilized and placed in one of two media formulations: McCown's Woody Plant basal salts with Murashige and Skoog vitamins or Gresshoff and Doy basal salts and vitamins. Both formulations had 3% sucrose, 0.6% agar, 0.89 µM 6-benzylaminopurine, and 100 mg/L benomyl. Growth responses observed varied by species and included leaf expansion, bud expansion, shoot production, and callus production among nine of the species.

Bud-Forcing and Tissue Culture Propagation of *Quercus* L.

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Introduction

- Oaks (*Quercus* L.) are valued globally for their strong economic, ornamental, and ecological contributions, but despite their importance, many species of *Quercus* are under threat from a wide range of global issues (Oldfield and Eastwood 2007).
- One method of saving threatened species is tissue culture using newly-flushed shoot tips (Kramer and Pence 2012).
- Natural shoot emergence in the spring is a narrow and somewhat unpredictable time window, but forcing bud break of cuttings can increase this window in a controlled environment (Vieitez et al. 1994).
- Two experiments were conducted to investigate bud-forcing and tissue culture propagation of *Quercus* species.

Experiment 1: Bud- Forcing

Objective

To determine the effectiveness of 6-benzylaminopurine (BAP), a cytokinin (hormone that promotes cell division), on bud break in 12 *Quercus* species

Materials and Methods

- **12 species of *Quercus***: *alba, bicolor, cerris, falcata, imbricaria, macrocarpa, macrocarpa* var. *macrocarpa, pagoda, palustris, rubra, texana,* and *variabilis*
- **Plant Material**: Terminal cuttings (10-33 cm long; 5-25 buds each) harvested Feb. 2015 (-16 to -5°C) in Kennett Square, PA, USA
- **Experimental Treatment:** Factorial design with 12 species, 3 BAP treatments (0 ppm, 100 ppm, and 500 ppm applied weekly by paint brush), and 3 replications (108 cuttings in total)
- **Conditions & Environment**: Erlenmeyer flasks with distilled water placed in a greenhouse (heat set point: 20°C; cooling set point: 26.5°C)

Experiment 2: Tissue Culture

Objective

To determine the responses of 12 North American *Quercus* species representing three sections on two different types of tissue culture media

Materials and Methods

- **12 North American species of *Quercus*, divided into taxonomic section:**
  Lobatae (black oaks): *arkansana*, *canbyi, graciliformis,* & *texana*
  Quercus (white oaks): *boyntonii, dumosa, engelmannii,* & *gambelii*
  Protobalanus (canyon live oaks): *chrysolepis, palmeri, tomentella,* & *vacciniifolia*
- **Explants:** Surface-sterilized leaf cuttings from young shoots of 38 trees from 9 US botanic gardens
- **Treatments (Media Formulations)**: 1. Lloyd & McCown's Woody Plant Medium basal salts (1980) with Murashige & Skoog vitamins (1962) and 2. Gresshoff & Doy basal salts and vitamins (1972), both with 3% sucrose, 0.6% agar, 0.89 μM BAP (cytokinin), and 100 mg/L benomyl (fungicide)
- **Experiment:** Factorial design with 12 species, 2 media treatments, and 6-27 replications, depending on material availability (419 explants in total)
- **Conditions & Environment**: Individual 25x150 mm culture tubes; 22.5-25.5°C ambient temperature; cool white fluorescent lighting at 20-25 μmol/m2/sec

Results

Responses varied by *Quercus* species and section and included leaf expansion, bud enlargement, shoot production, and callus production among nine of the species.

Table 1: Growth responses to growing media of each species. GD = Gresshoff & Doy Media; WP = Lloyd & McCown's Woody Plant/Murashige & Skoog Media; (-) = no response.

Quercus Section/Species	No. of Explants	% Exhibiting: Contamination		Expansion of Existing Leaves		Enlargement of Buds		Development of New Shoots		Callus	
	Media	GD	WP	GD	WP	GD	WP	GD	WP	GD	WP
Lobatae	192	24.5	28.1	1.6	1.6	-	1.0	0.5	1.0	1.0	0.5
arkansana	54	14.8	13.0	5.6	5.6	-	1.9	1.9	3.7	-	-

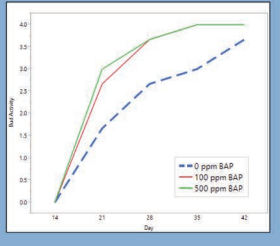
development and 4 = target for shoot tip micropropagation (Figure 1)

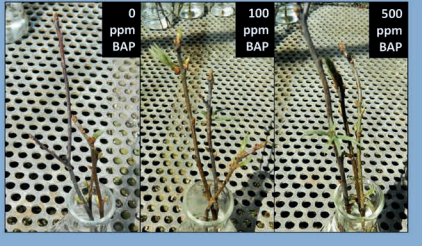
Figure 1: Bud activity evaluation scale

Results

BAP treatment at 100 or 500 ppm significantly increased the rate of bud break and shoot elongation for four of the *Quercus* species: *imbricaria* (Figures 2 & 3), *macrocarpa, pagoda,* and *variabilis*.

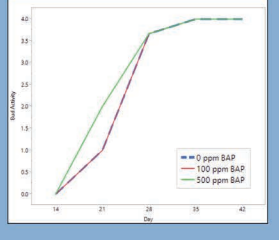
Figures 2 & 3: *Q. imbricaria* (example sp. significantly affected by BAP treatment): mean bud activity (L) and cuttings, day 35 (R)

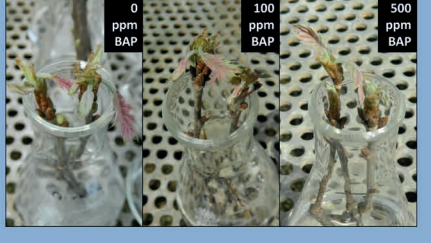




Little to no significant effect from BAP application on the remaining eight *Quercus* species: *alba, bicolor, cerris, falcata, macrocarpa* var. *macrocarpa, palustris, rubra* (Figures 4 & 5), and *texana.*

Figures 4 & 5: *Q. rubra* (example sp. not significantly affected by BAP treatment) – mean bud activity (L) and cuttings, day 35 (R)





Key Findings

- The effect of the cytokinin, BAP, on *Quercus* bud-forcing varied by species.
- All species except *alba, bicolor*, and *pagoda* reached stage 4 with all treatments.
- Main conclusion: forcing bud break without BAP application is a viable option, but the rate may be enhanced with some *Quercus* species by the application of BAP.

texana	36	5.6	13.9	-	-	-	-	-	5.6	-
Quercus	140	22.1	25.0	0.7	4.3	-	5.0	-	1.4	2.1	2.1
boyntonii	30	6.7	16.6	3.3	6.7	-	-	-	-	-	-
dumosa	18	22.2	27.8	-	-	-	-	-	-	16.7	11.1
engelmannii	44	9.1	11.4	-	2.3	-	13.6	-	-	-	2.3
gambelii	48	43.8	41.7	-	6.3	-	2.1	-	4.2	-	-
Protobalanus	87	18.4	23.0	3.4	4.6	2.3	1.1	-	-	-	-
chrysolepis	12	8.3	25.0	-	-	-	-	-	-	-	-
palmeri	20	10.0	15.0	-	-	-	-	-	-	-	-
tomentella	30	20.0	20.0	10.0	13.3	-	-	-	-	-	-
vacciniifolia	25	28.0	32.0	-	-	8.0	4.0	-	-	-	-

Figure 6: Images of *Quercus* explant growth responses: 1) expansion of existing leaves, 2) bud enlargement, 3) new shoot development, & 4) callus production

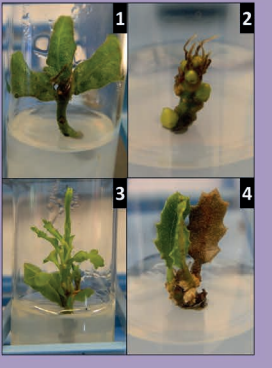
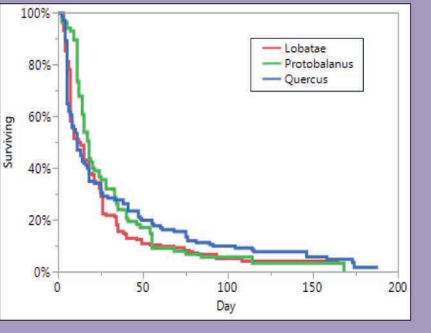


Figure 7: Percent of *Quercus* explants surviving over time by section



Key Findings

- The overall growth response rate was low among all species, but the Quercus section exhibited the highest response rate compared to Lobatae and Protobalanus.
- The most common growth response was expansion of existing leaves.
- Explants growing on the Lloyd & McCown/Murashige & Skoog media showed a slightly longer survival time than those on the Gresshoff & Doy formulation, but overall mortality was high across all media and species.
- Main conclusion: additional research into media formulations and sterilization methods is especially necessary to make oak tissue micropropagation a viable option for conservation purposes.

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