CLONAL PROPAGATION OF OAK HYBRIDS USING A MODIFIED LAYERING TECHNIQUE

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ABSTRACT

Oak (*Quercus*) hybrids were created using over 40 diverse parent species. The developed hybrids were used as stock plants and asexually propagated annually over four years. This was done to measure the effectiveness of a modified stool bed layering technique on diverse members of the oak genus, and this study is part of a long-term project to select superior urban-tolerant oak hybrids for introduction as named cultivars into the nursery industry. The number of shoots produced by a stock plant each year and the probability for those shoots to root were found to vary between different maternal parent species. Results suggest the shoots of the hybrids that are the progeny of rhizomatous shrub *Quercus* spp. are more likely to develop roots when propagated using the described technique. This article also identifies and describes in detail a reliable technique to clone oaks.

INTRODUCTION

Oaks (*Quercus* spp.) are noted for their sexual infidelity, and natural hybrids commonly occur wherever two interfertile species from the same subgenera grow near each other (Sternberg, 2000). This tendency to hybridize is a useful trait that may be utilized by plant breeders to create hybrids with desirable characteristics such as attractive fall color, tolerance to site conditions, and hybrid vigor. Increasingly, a number of purported hybrid oak selections are becoming available in the nursery industry (JFSchmidt) (Dirr, 2010).

Until recent years, relatively few selections of oaks have been made due to the difficulty involved in asexually propagating members of this genus by conventional methods. Oaks are notoriously difficult to bud or graft successfully, which compounds the problem of producing superior selections. Recently, a modified layering technique that incorporates juvenile shoots, etiolation, and treatment with indolebutyric acid (IBA) has shown promise in the asexual propagation of *Quercus* species (Amissah & Bassuk, 2009) (Hawver & Bassuk, 2000). A study examining the usefulness of this propagation method on numerous hybrid oaks was conducted at the research farms of Cornell University, in Ithaca, New York. The hybrids used represent crosses of over 40 diverse parent species, most of which are in the white oak group (subgenus *Quercus*). A previous study has shown that the rooting success of oaks propagated asexually using a similar method can differ between species (Amissah & Bassuk, 2007), and preliminary research suggests that the propagation success of individual hybrid oaks would vary depending on the parental species involved in each cross (Gao, 2011).

The objective of this research was to measure the effectiveness of the modified stool bed layering technique on diverse members of the genus *Quercus*, as well as to provide insight into which, if any, parent species consistently yielded hybrid progeny that could be successfully propagated. Additionally, this article identifies and describes in detail a reliable technique to clone oaks. This experiment is part of a long-term project underway at Cornell's Urban Horticulture Institute to select superior urban-tolerant oak hybrids for introduction as named cultivars into the nursery industry. Along with developing a reliable method to clone oaks, the project currently involves screening the developed hybrids for tolerance to alkaline soils (a common issue in urban landscapes) and making long-term observations on growth and landscape performance.

MATERIALS AND METHODS

Trees used in this study were hybrids grown from seed that were developed by Peter Podaras at Cornell University in Ithaca, New York (Podaras & Wells, 2008). During the years 2004, 2005, and 2006, controlled crosses were made by pollinating seven species of oak trees growing on the Cornell University campus with pollen from 36 species that are native throughout North America, Europe, and Asia. Table 1 details the number of hybrid seedlings developed from each cross. These seedlings were grown in containers for several years, and during the spring of 2008, 345 unique oak genotypes were planted out in a field of Arkport sandy loam with a pH of 6.2 for future use as stock plants. Starting in spring of 2009, and repeated yearly through 2012, these field-grown stock plants were propagated each year using a modified version of the oak propagation protocol developed by Amissah and Bassuk (2009).

Table 1. Hybrid crosses performed and the resulting number of acorn-grown genotypes.

Maternal Parent	Paternal Parent	Number of Genotypes
Q. bicolor	OPEN POLLINATED	7
	Q. sp. (unidentified species)	9
	Q. ×*bebbiana*	5
	Q. muehlenbergii × *Q. robur*	6
	Q. affinis	3
	Q. aliena	27
	Q. austrina	4
	Q. chapmanii	4
	Q. dentata	7
	Q. fabri	14
	Q. fruticosa	4
	Q. fusiformis	2
	Q. gambelii	5
	Q. geminata	2
	Q. glauca	1
	Q. graciliformis	11
	Q. libani	3
	Q. lyrata	2
	Q. macranthera	1
	Q. minima	5
	Q. mongolica var. *grosserata*	3
	Q. muehlenbergii	22
	Q. myrsinifolia	19
	Q. phillyreoides	7
	Q. polymorpha	4
	Q. robur	14
	Q. rugosa	13
	Q. spinosa	2
	Q. turbinella	6
	Q. vaseyana	6

Table 1 *continued*.

Maternal Parent	Paternal Parent	Number of Genotypes
Q. 'Ooti'	*Q. fusiformis*	1
Q. ×*warei* 'Long'	*Q.* ×*comptoniae*	17
	Q. ×*warei* 'Long'	8
Q. gambelii x *macrocarpa*	Q. ×*comptoniae*	2
	Q. lyrata	2
Q. macrocarpa	OPEN POLLINATED	12
	Q. ×*comptoniae*	1
	Q. ×*undulata*	4
	Q. fusiformis	2
	Q. gambelii	6
	Q. geminata	1
	Q. lyrata	4
	Q. macrocarpa	1
	Q. michauxii	3
	Q. minima	1
	Q. prinoides	13
	Q. turbinella	1
Q. montana	*Q. geminata*	2
	Q. lyrata	1
Q. muehlenbergii	*Q.* ×*comptoniae*	1
	Q. aliena	2
	Q. fusiformis	16
	Q. geminata	2
	Q. lyrata	3
	Q. michauxii	3
	Q. minima	4
	Q. muehlenbergii	1
	Q. prinoides	6
	Q. virginiana	7

2009 Propagation. Before bud break in May of 2009, each hybrid oak stock plant was cut back, leaving an 8 cm (3 in) stump. After 2-3 weeks, upon evidence of epicormic bud swelling on the stump, the plants were etiolated. To achieve etiolation, each stump was covered with an inverted #2 container wrapped in heavy-duty aluminum foil to reduce heat accumulation. A brick was placed on top of each container to secure it in place.

When the newly expanded shoots reached approximately 12 cm (4.7 in) in length, which took ~7 days, the pots used for etiolation were removed. At that time, any shoots less than 5 cm (2 in) in length were removed, and the basal 3 cm (1.2 in) section of the new shoots was sprayed with a solution of 8,000 ppm Indol-3-butyric Acid (IBA) dissolved in 98% aqueous ethanol.

After the IBA solution dried (~10-15 minutes after treatment), a #2 bottomless pot wrapped with light-reflective aluminum was placed over each stock plant so that it rested on the surface of the soil. The bottomless pots were filled with pre-moistened PRO-MIX[1] (a peat-based growing medium by Premier Tech Horticulture) to cover the treated shoot bases while leaving the growing tips of the shoots exposed. The tips of the shoots, while coming out of the planting medium, were still contained within the headspace of the bottomless pot.

The openings of the pots were then covered with white plastic perforated with two cuts to reduce humidity. This was done to protect and temporarily shade the etiolated shoots and allow them to gradually acclimate to the increased irradiance levels while greening up. After 2 weeks, the white plastic was removed and the shoot tips were exposed to full sun conditions. Since the stock plants grew at different rates, all treatments were completed in late July of 2009.

The plants were then allowed to grow. Over the growing season, overhead irrigation was used when necessary to keep the medium in the bottomless pots moist. Additional medium was added as the shoots elongated until it entirely filled the bottomless pots. Throughout the summer, the lengthening shoots were pruned back to approximately 60 cm (23.6 in) to reduce ultimate shoot height.

The plants were allowed to grow until early November when the bottomless pots and the growing medium were removed, and the rooted and unrooted shoots were harvested from the stock plants. Shoots that had developed roots were potted up with PRO-MIX in #1 containers. During harvesting, the number of total shoots and the number of rooted shoots were recorded for each stock plant. The potted oaks were thoroughly watered, then placed in an unheated covered overwintering structure for winter dormancy.

2010 Propagation. The propagation procedure was repeated in 2010 with a few adjustments. The IBA concentration was reduced from 8,000 to 6,000 ppm in 98% aqueous ethanol, and it was applied with a soft paintbrush instead of a spray bottle. Rather than using white plastic for shading and the acclimatization of the etiolated shoots to full sun, silver-colored, light-reflective metal mesh trashcans approximately 30 x 30 x 35 cm (12 x 12 x 14 in) in size were used to cover and shade the shoots following etiolation. After the shoots gradually greened up under the shade for approximately 1 week, the trashcans were removed.

2011 & 2012 Propagation. A few adjustments were made to the 2010 procedure for use in both 2011 and 2012. Rather than harvesting and potting up the rooted shoots in the fall, they were left until the spring, allowing the shoots to undergo dormancy while still attached to the stock plants. The shoots were then harvested before bud break. Also, rather than using bottomless #1 pots wrapped in aluminum foil for the layering step of the procedure, a section of white PVC pipe, approximately 15.25 cm tall with a diameter of 16.5 cm (6 x 6.5 in), was employed. The IBA concentration was also increased back to 8,000 ppm from 6,000 ppm. Figure 1 illustrates the procedure used during these years and Table 2 details the suggested protocol for rooting *Quercus* spp. using this technique.

[1] Main components: Canadian sphagnum peat moss (75-85 % / vol.), perlite, vermiculite, dolomitic & calcitic limestone (pH adjuster), macronutrients, micronutrients, wetting agent

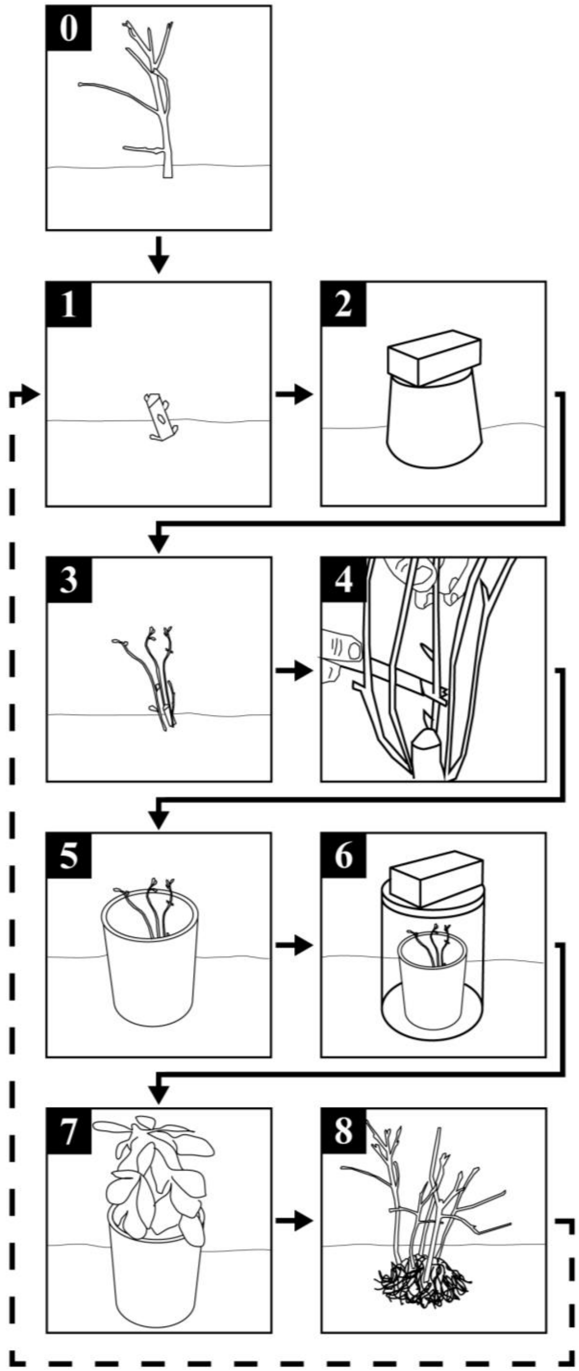

Figure 1. Field layering procedure for *Quercus spp*.
The oaks are planted in a field and grown for one to two years for future use as stock plants (0). In spring, while the plant is dormant, it is cut back to an 8cm stump. When the buds begin to swell (1), the plant is covered for etiolation (2). The container used for etiolation is removed 1 week later (3). At this time, IBA is painted onto the bases of the new shoots (4). A bottomless container is then placed over the stock plant and filled with a soilless medium (5). The plant is then covered with a metal mesh trashcan to temporarily shade the plant as it becomes acclimated to full sun (6). After the trashcan is removed, the plant is allowed to grow all season and the medium in the bottomless pot is

kept moist (7). The following spring, the pot and medium are removed, and the shoots — which hopefully have rooted — are then harvested from the stock plant (8). The entire procedure can be repeated the following year.

Table 2. Suggested protocol for rooting *Quercus* spp.

Time		**Action**
Time 0	Immediately prior to bud break	Stock plants cut back to ~8cm above soil
Week 2-3	Buds start expanding	Stumps covered with #2 containers that are wrapped in aluminum foil and secured with brick for etiolation
Week 3-4	~7 days later	Containers removed. IBA applied to base of newly developed shoots. Bottomless pot placed over shoots and filled with soilless medium. Stock plant covered with a metal mesh trashcan for shading
Week 4-5	~7 days later	Trashcans removed
Throughout growing season		Plants pinched back to ~60cm, and medium is kept moist to encourage rooting
Following spring, before bud break		Rooted shoots harvested. Protocol can be repeated in following years

Statistical Analysis. Statistical analysis of the data was done using SAS statistical software (SAS Institute Inc.) and the unit for analysis was a single shoot. Due to the large number of paternal parent species, and the fact that many hybrid crosses were represented by only a few genotypes, the data was pooled by maternal parent species, and the data from all four years were combined for analysis. Table 3 summarizes the number and diversity of stock plants (genotypes) when sorted by maternal parent species. The effect of the maternal parent species on the total number of shoots produced annually by a stock plant and the effect on the probability of a shoot to produce roots were studied.

Table 3. Summary of hybrid crosses sorted by maternal parent species

Maternal Parent	**Number of Unique Hybrid Crosses**	**Number of Unique Genotypes**
Q. bicolor	30	218
Q. gambelii × *Q. macrocarpa*	2	4
Q. macrocarpa	12	49
Q. montana	2	3
Q. muehlenbergii	10	45
Q. 'Ooti'	1	1
Q. ×*warei* 'Long'	2	25

In order to study the effect of the maternal parent species on the number of shoots produced, a generalized linear mixed model using a Gauss-Hermite quadrature method with a Poisson distribution and log link was employed. For studying the effect of maternal parent on the likelihood of a shoot to produce roots, a generalized linear mixed model with a binomial distribution and logit link was used. For both regression analyses, the combination of parents (hybrid species) was controlled for as a random effect. Least squares means was used to estimate the number of shoots a stock plant produced annually and the probability of a produced shoot to root based on the maternal parent. The Tukey-Kramer method was used to compare differences of maternal parent least square means with differences being considered significant when $P < 0.05$.

RESULTS AND DISCUSSION

Shoot Production. The mean number of shoots produced annually by stock plants varied significantly between maternal parent species (Table 4). The mean number of shoots produced annually was highest in the hybrid with *Quercus* 'Ooti' as a maternal parent (8.9 shoots/year) and lowest in *Quercus bicolor* (4.8 shoots/year) (Table 5). The Tukey-Kramer HSD test reveals that *Q. bicolor* and *Q. macrocarpa* values are significantly different ($P < 0.05$) when compared to *Quercus* 'Ooti' and *Q. muehlenbergii* (Figure 2). It should be noted that the limited number of stock plants with *Q.* 'Ooti', *Q. montana*, and *Q. gambelii* × *Q. macrocarpa* as a maternal parent (Table 3) resulted in high standard error values of over 1.0 for these three species (Table 5).

Table 4. Statistics of number of shoots produced by stock plants by maternal parent.

Effect	Num DF	Den DF	*F* Value	Prob > *F*
Maternal Parent	6	52	2.96	0.0146

Table 5. Estimated annual number of shoots produced by maternal parent.

Maternal Parent	Estimate Number of Shoots	Standard Error	Prob > |t|
Q. bicolor	4.8	0.2	< 0.0001
Q. gambelii × *Q. macrocarpa*	6.7	1.1	< 0.0001
Q. macrocarpa	5.2	0.4	< 0.0001
Q. montana	6.2	1.1	< 0.0001
Q. muehlenbergii	6.2	0.4	< 0.0001
Q. 'Ooti'	8.9	2.2	< 0.0001
Q. ×*warei* 'Long'	5.4	0.7	< 0.0001

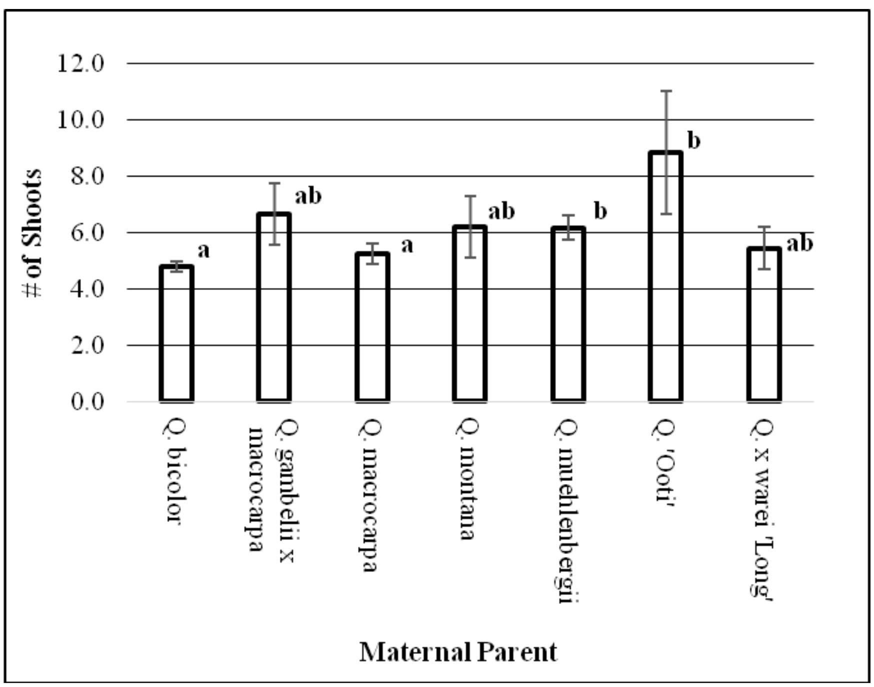

Figure 2. For the yearly number of shoots produced by stock plants, estimates based on the maternal parents were found to be significantly different ($P < 0.05$). Different letters indicate significant differences ($P < 0.05$, Tukey-Kramer HSD test). Error bars represent calculated standard error.

Rooting Probability. The probability for a shoot to develop roots varied significantly between the maternal parent species. Table 6 summarizes the statistical analysis. The probability for a shoot to develop roots was highest in the hybrids with *Q. gambelii* × *Q. macrocarpa* as a maternal parent (62.3%) and lowest in *Q. muehlenbergii* progeny (7.0%) (Table 7). The Tukey-Kramer HSD test reveals that values for *Q. bicolor* , *Q. gambelii* × *Q. macrocarpa*, and *Q. muehlenbergii* were all significantly different ($P < 0.05$) from each other (Figure 3). Similar to the statistical analysis of the number of shoots produced annually, the limited number of stock plants with *Q.* 'Ooti', *Q. montana*, and *Q. gambelii* × *Q. macrocarpa* as a maternal parent resulted in high standard error values of over 12%. *Q.* ×*warei* 'Long' also had a high standard error value over 12%. Additionally, these four species had Prob > |t| values greater than 0.05.

The relatively high rooting probability associated with the maternal parent *Q. gambelii* × *Q. macrocarpa* might be related to the fact that *Q. gambelii*, the Gambel Oak, is a shrubby species native to the southwestern United States that spreads by rhizomes (Grimshaw & Bayton, 2009). *Q.* 'Ooti' is also associated with high rooting probability. *Q.* 'Ooti' is a cultivar of difficult to determine origins and is supposedly a selection of the complex hybrid *Q. robur* × *Q. macrocarpa* × *Q. muehlenbergii* (Pavia Nursery). Because *Q.* 'Ooti' is only represented by a single cross and genotype (Table 3), the high rooting probability might be connected to the paternal parent species involved ~ *Q. fusiformis*, the Texas Live Oak. This is another shrubby species from the southwestern United States that spreads by rhizomes (Grimshaw & Bayton, 2009). On a related note, for the long-term goals of this project, it is of interest to observe the mature growth habit of all of the developed oak hybrids, especially those that involve *Q. gambelii, Q. fusiformis* or any of the other rhizomatous shrub species used.

Table 6. Statistics of the probability of a shoot to root based on maternal parent

Effect	**Num DF**	**Den DF**	***F* Value**	**Prob > *F***
Maternal Parent	6	52	7.82	< 0.0001

Table 7. Estimates for probability of shoots to root by maternal parent

Maternal Parent	**Estimate of Rooting Probability**	**Standard Error**	**Prob > |t|**
Q. bicolor	19.4%	2.2%	< 0.0001
Q. gambelii × *Q. macrocarpa*	62.3%	12.7%	0.359
Q. macrocarpa	27.4%	4.7%	0.0001
Q. montana	33.7%	13.0%	0.2494
Q. muehlenbergii	7.0%	1.8%	< 0.0001
Q. 'Ooti'	60.0%	18.8%	0.6083
Q. ×*warei* 'Long'	48.8%	12.7%	0.9229

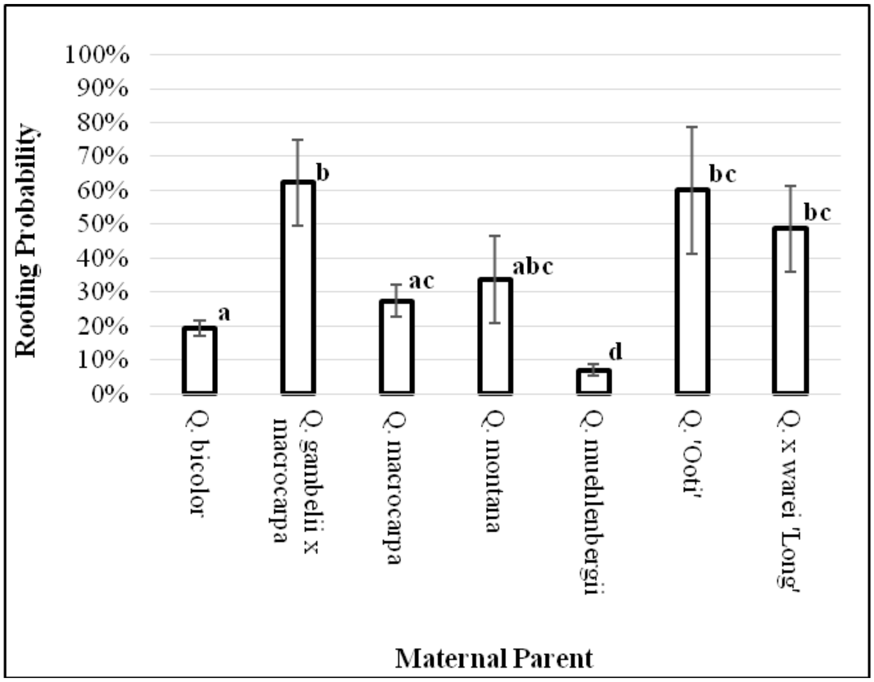

Figure 3. For the probability of shoots to produce roots, estimates based on the maternal parents were found to be significantly different ($P < 0.05$). Different letters indicate significant differences ($P < 0.05$, Tukey-Kramer HSD test). Error bars represent calculated standard error.

Conclusions. These results have shown that the described propagation method can be utilized to clone genetically diverse members of the genus *Quercus*. However, out of the 345 unique genotypes originally involved in the study, 88 of the stock plants had died by 2012, and only 235 of them produced rooted shoots at any point during the four years. This suggests that there is more to learn about developing a successful asexual propagation method for diverse *Quercus* species. Moreover, the number of rooted shoots produced annually by a stock plant will likely need to increase for this method to become a viable commercial propagation practice. It is expected that the described propagation method, which has proven successful on a diverse range of oaks, could be further optimized to have greater success on a given species, hybrid, or cultivar of interest.

The results of this study provide guidance to plant breeders interested in creating oak hybrids and suggest that certain maternal parents are preferable to others if the goal is to create hybrids that can be asexually propagated in good numbers using the described method. Additionally, the findings will be used to select from the hybrids the genotypes that are highly propagable for future observation. With completion of this study, Cornell's Urban Horticulture Institute is one step closer to realizing the goal of selecting and introducing named cultivars of superior urban-tolerant oak hybrids.

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