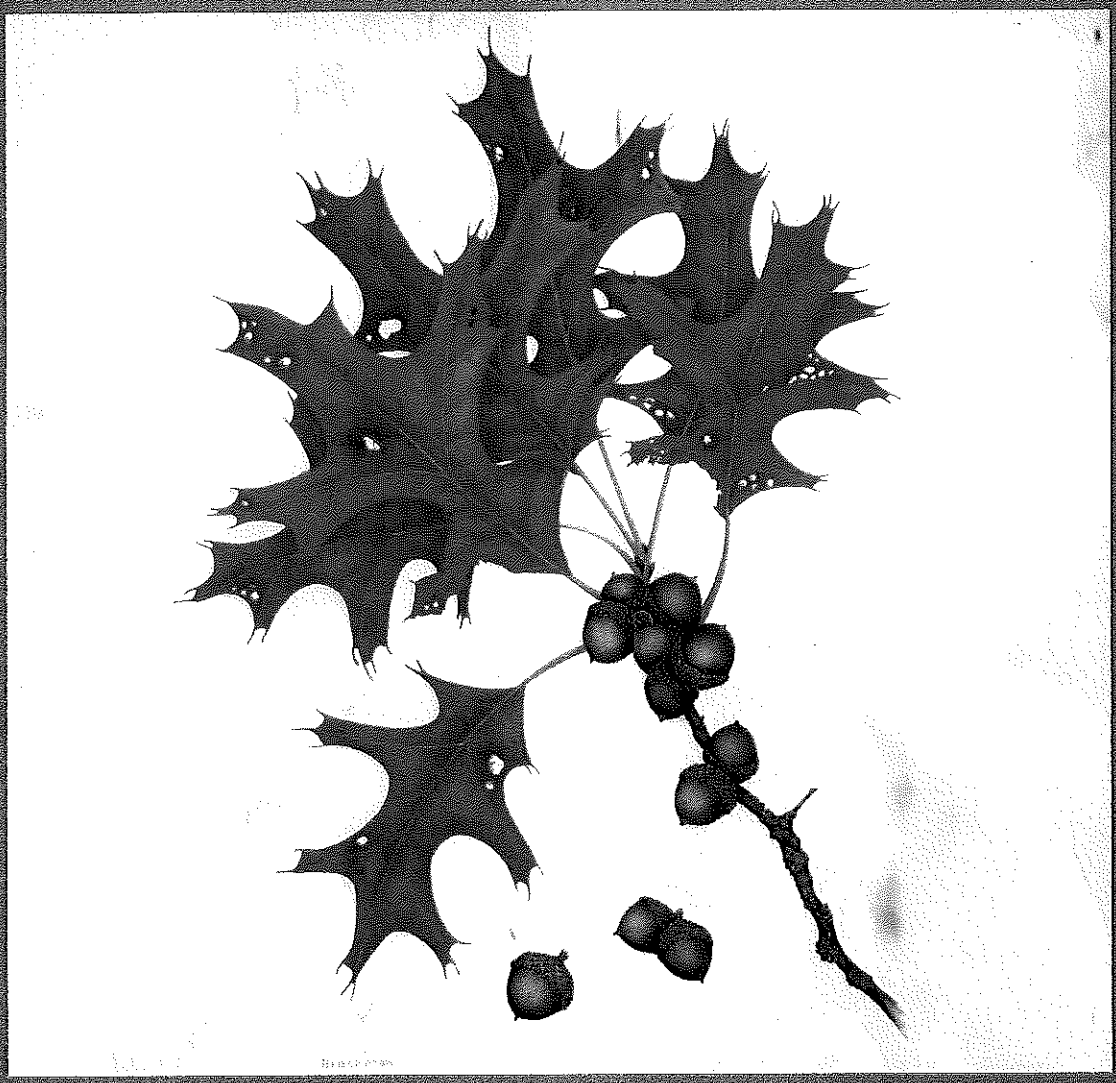


International Oaks

The Journal of the International Oak Society

Proceedings

8th International Oak Society Conference
October 18-21, 2015

Issue No. 27/ 2016 / ISSN 1941-2061

Ecology Resources 16:254-

)mic regions differentiating

A. Kremer. 2004. Genome
l *Q. petraea* (Matt.) Liebl.).

.iebl. *Annales des Sciences*

:rungen. *Allgemeine Forst-*

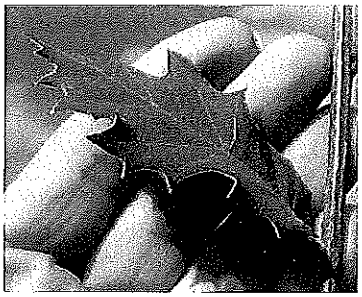
:haracterization of genomic
Reporter 31: 231-239.

Rombauts, A. Salamov, J.
er, V. Busov, M. Campbell,
Cronk, R. Cunningham, J.
ılting, B. Ellis, K. Gendler,
(elariutta, B. Henrissat, D.
Cangasjarvi, J. Karlsson, C.
Locascio, Y. Lou, S. Lucas,
'eter, R. Philippe, G. Pilate,
., J. Schrader, B. Segerman,
Nessler, G. Yang, T. Yin, C.
wood, *Populus trichocarpa*

h-gene-flow. *Philosophical*

inese oaks. *Plos One* 5: 15.
suggests interspecific gene

).J. Yun, and R.A. Bressan.
of the National Academy of



Asexual Propagation of Oak Hybrids: Our Progress, and the Challenges of Producing Clonal Plants

Nina L. Bassuk*, Bryan R. Denig, and Miles Schwartz Sax
School of Integrative Plant Science, Urban Horticulture Institute, Cornell University
Ithaca, NY 14853, USA
*corresponding author: *nlb2@cornell.edu*

ABSTRACT

One research focus of the Urban Horticulture Institute (UHI) at Cornell University involves the selection, evaluation, and propagation of superior plants for urban environments. In an effort to develop oaks suitable for urbanized environments, the UHI developed approximately 345 genotypes of hybrid oaks, consisting of approximately 55 unique crosses of over 40 diverse parent species in 2004-2006. These diverse hybrids have the promise of increased vigor and better adaptation to urban stresses such as alkaline soil, flooding, drought, and pests. Since 2004, we have been working on propagating, evaluating, and selecting from these hybrids superior oaks for urban landscapes. The ultimate goal of this long-term project is to introduce these superior hybrid oak selections into the nursery trade as named cultivars. A reliable asexual propagation method for oaks is crucial for meeting our goals, and the UHI has been pursuing such a method for almost 20 years. We have been successful in utilizing a modified stool bed layering technique, but the yield is too low for commercial applications. To overcome this challenge, tissue culture techniques are currently being developed as a means of rapid multiplication of large numbers of clonally propagated hybrid oaks.

Keywords: propagation, asexual, tissue culture, urban landscapes, stool bed layering

The case for clonal propagation

Trees in the genus *Quercus* are known for their longevity, ability to tolerate stress, and beautiful landscape characteristics. Because of these attributes, oaks have consistently been prized by nursery professionals and municipal arborists. For the most part, the oaks available today in the nursery trade are grown from seed. There are many reasons why growing oaks from acorns may be a preferable method of propagation, especially if the goal is to preserve genetic diversity. Still, there are instances when clonal (asexual) propagation methods, such as taking cuttings, layering, grafting, or tissue culture, are preferable.

In some cases, such as street tree plantings, predictability is needed. Choosing an oak that will be tolerant of the stresses of an urban landscape becomes more difficult when acorn-grown trees can vary dramatically in their characteristics. For example, acorns collected from a tree that has shown tolerance to alkaline soils (a common issue in urban landscapes), are not necessarily going to grow into plants that possess the same characteristic. Also, given that many oaks readily hybridize, one can never be certain which characteristics an acorn-grown tree will possess. While this element of surprise is one of the thrills of growing oaks from seed, many times there is a need to propagate oaks asexually. Reasons for clonal propagation might include multiplying individuals of horticultural merit, preserving the genetics of trees of special interest (such as F1 hybrids or historic trees), and aiding in the conservation of endangered *Quercus* species.

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. Delayed graft incompatibility is a common issue, and instances of grafts failing after seven years of perfect health have been recorded. However, the grafting work being done by Dirk Benoit of Pavia Nurseries in Belgium and other IOS members suggests that successful grafting of superior oak selections will become increasingly more common in the near future.

For nearly 20 years Cornell University's Urban Horticulture Institute has been doing research on various methods of oak asexual propagation: cuttings, layering, and tissue culture. We have been successful in developing a stool bed layering technique that can be used to produce rooted shoots, but the yield is too low to be commercially viable. This low propagation rate also limits our ability to set up trialing experiments for oak genotypes in which we are interested. Because of this, we are currently working on developing tissue culture techniques as a means for rapid multiplication of large numbers of clonally propagated oaks.

Our interest in oak hybrids

There are many ways to identify and select trees that will survive in difficult urban landscapes. One method for increasing the usefulness of certain trees involves taking advantage of intraspecific variation within the population of the species of interest, and selecting for cultivars that are tolerant of urban stresses such as alkaline soils (Steiner 1980). This is a worthwhile approach for oaks, as many native oak species are distributed over a wide range of North America, and considerable intraspecific variation has been shown in a number of species (Kriebel 1993).

Rather than making selections from genetically varied native populations to obtain oaks that possess certain characteristics, another strategy for expanding the palette of oaks suitable for urban landscapes involves creating deliberate hybrid crosses. The genus *Quercus* is noted for the propensity of many of its species to readily hybridize with others within their own taxonomic section. Deliberately creating hybrids that combine traits from species that do not share the same geographic range can increase the chance of finding alkaline-tolerant individuals. Hybrids could potentially combine the trait of a tolerance to alkaline soils (found particularly in species native to the Southwestern United States) with other desirable landscape characteristics such as cold hardiness, attractive fall color, good branching habit, tolerance to various soil moisture conditions, and heterosis (hybrid vigor). Such trees have the potential to expand the palette of oaks that will thrive in temperate urban landscapes, and their use could potentially increase the biodiversity and resiliency of the urban forest. Not surprisingly, in the last few years, the nursery industry has seen an increased number of purported hybrid oak selections become available (Dirr 2010).

Our hybrids

From 2004 to 2006, Peter Podaras, working with the Urban Horticulture Institute at Cornell University, made controlled hybrid crosses by pollinating seven species of oak trees growing on the Cornell University campus in Ithaca, New York, USA (USDA winter Hardiness Zone 5b) with pollen from 36 species that are native throughout North America, Europe, and Asia (Podaras and Wells 2008). Collectors and arboreta were kind enough to send pollen to Cornell for this project, and our thanks go out to all who helped make these crosses possible.

The maternal parents (the trees that were pollinated and produced the hybrid acorns) were composed of these taxa: *Quercus* 'Ooti' (provisionally accepted; purportedly a cultivar of *Q. robur* L. × *Q. macrocarpa* Michx.× *Q. muehlenbergii* Engelm.), *Q. gambelii* Nutt. × *Q. macrocarpa* Michx., *Q. bicolor* Willd., *Q. macrocarpa* Michx., *Q. montana* Willd., *Q. muehlenbergii* Engelm., and *Q.* ×*warei* T.L. Green & W. J. Hess. The pollen parents were much more diverse, and are detailed in Table 1.

While we are fairly confident in the identities of the maternal parents of our hybrids, there is the possibility that the paternal parents differ from what we have recorded. Issues such as contaminated pollen, mislabeled and misidentified trees in collections, and general human error mean that some of our hybrids might not be exactly what we think they are. For example, we are a bit suspicious of our plants that are purportedly intersectional crosses, such as what we are calling a hybrid between *Q. bicolor* (section *Quercus*) and *Q. graciliformis* C.H. Mull. (section *Lobatae*).

The hybrid seedlings resulting from these crosses were planted out in Cornell's research fields in 2008 for use as stock plants. They were subsequently coppiced each spring and clonally propagated during the 2009, 2010, 2011, and 2012 growing seasons using a stool bed layering technique. Propagating these plants asexually allowed us to set up trialing experiments to help us determine which of these hybrid oak genotypes were good candidates for introduction as named cultivars. A greenhouse study (Denig et al. 2014) comparing the performance of these hybrids in alkaline (pH 8) and acidic (pH 6) growing media has allowed us to single out individual genotypes that are likely tolerant of the highly alkaline soils common in urban landscapes.

Scientific Name	Common Name
Quercus muehlenbergii Engelm. × *Q. robur* L.	
Quercus ×*bebbiana* C.K. Schneid. [Q. *alba* L. × *Q. macrocarpa* Michx.]	Bebb's oak
Quercus ×*comptoniae* Sarg. [*Q. lyrata* Walter × *Q. virginiana* Mill.]	Compton's oak
Quercus ×*undulata* Torr.	wavy-leaf oak
Quercus ×*warei* T.L. Green & W.J. Hess [Q. *robur* L. × *Q. bicolor* Willd.]	
Quercus affinis Scheidw.	
Quercus aliena Blume	oriental white oak
Quercus austrina Small	bluff oak
Quercus chapmanii Sarg.	Chapman oak
Quercus dentata Thunb.	daimyo oak
Quercus fabri Hance	
Quercus fruticosa Brot.[1]	Lusitanian oak
Quercus fusiformis Small	Texas live Oak
Quercus gambelii Nutt.	Gambel oak
Quercus geminata Small	sand live Oak
Quercus glauca Thunb.	Japanese blue oak
Quercus graciliformis C.H. Mull.	slender oak
Quercus libani G. Olivier	Lebanon oak
Quercus lyrata Walter	overcup oak
Quercus macranthera Fisch. & C.A. Mey. ex Hohen.	Caucasian oak
Quercus macrocarpa Michx.	bur oak
Quercus michauxii Nutt.	swamp chestnut oak
Quercus minima (Sarg.) Small	Dwarf Live Oak
Quercus mongolica var. *grosserata* (Blume) Rehder & E.H. Wilson	mizu-nara Japanese Oak
Quercus muehlenbergii Engelm.	chinkapin oak
Quercus myrsinifolia Blume	Chinese evergreen Oak
Quercus phillyreoides A. Gray	ubame Oak
Quercus polymorpha Schltdl. & Cham.	Mexican white oak
Quercus prinoides Willd.	dwarf chinkapin oak
Quercus robur L.	English oak
Quercus rugosa Née	net-leaved Oak
Quercus spinosa David	
Quercus turbinella Greene	Sonoran scrub oak
Quercus vaseyana Buckley	sandpaper oak
Quercus virginiana Mill.	southern live oak

Table 1/ Pollen parent species utilized in creating hybrid oaks.

1. A synonym of *Q. lusitanica* Lam.

Cloning via

In the simp
ground in the
giving the ne
from Cornell
of our stool b
summarize th
adventitious r

For use as
approximately
break, each st
tall. After 2-3
#2 plastic pot
placed over th

Once the et
are removed.
in 90% aqueo
original stump
of 15 cm, is th

A moist, lig
in the PVC cy
shoots remain
is placed ove
shoots to dire
grow, more sc
is kept moist,

The follow
rooted and ur
shoots, the en

This stool h
over four gro
number of sh
plant. The res
findings of th
shrubby, rhiza
likely to prod

Our stool b
from a large r
a single roote
plants, only 2
those that hav
stock plant ha
produced by
shoots is muc

Cloning via stool bed layering

In the simplest terms, stool bed layering involves cutting a stock plant down to the ground in the spring, and mounding soil around the base of the new shoots that arise, giving the new stems the opportunity to develop their own roots. Past research articles from Cornell University's Urban Horticulture Institute more fully describe the particulars of our stool bed layering technique (Amissah and Bassuk 2009; Denig et al. 2013). To summarize the research, the success of our method relies on three factors that enhanced adventitious root production: juvenility, etiolation, and indolebutyric acid.

For use as stock plants, our hybrid oak seedlings were planted in a field, and spaced approximately 2 m apart. The actual procedure begins in the early spring. Before bud break, each stock plant is cut nearly to the ground, leaving a stump approximately 8 cm tall. After 2-3 weeks, when the buds on the remaining stump just begin to swell, an inverted #2 plastic pot wrapped in heavy-duty aluminum foil (to reduce heat accumulation), is placed over the stump to etiolate the new shoots that will arise from the swollen buds.

Once the etiolated shoots grow to approximately 8-10 cm tall, the pots used for etiolation are removed. At this time, a solution of 8,000 ppm Indole-3-butyric Acid (IBA) dissolved in 90% aqueous ethanol is then painted onto the basal 3 cm of each stem proximal to the original stumps. A section of white PVC pipe, approximately 15 cm tall with a diameter of 15 cm, is then placed over the etiolated stems so that it rests on the ground.

A moist, lightweight, soilless medium made up primarily of peat is then gently placed in the PVC cylinder so that the bases of the etiolated stems are covered. The tops of the shoots remain above the medium. Immediately after this, a silver-colored mesh trashcan is placed over the PVC cylinder and shoots. This allows us to gently acclimatize the shoots to direct sunlight. After a week, the mesh trashcans are removed. As the stems grow, more soilless mix is added to the cylinders. During the growing season, the medium is kept moist, and stems are pinched back if they grow taller than 40 cm.

The following spring, the PVC cylinders and growing medium are removed, and the rooted and unrooted shoots are harvested from the stock plant stumps. After harvesting shoots, the entire propagation procedure can be repeated right away.

This stool bed layering technique has been repeated on all of our hybrid oak genotypes over four growing seasons (2009, 2010, 2011, and 2012), with data collected on the total number of shoots produced and the total number of rooted shoots produced by each stock plant. The results of this research have been published (Denig et al. 2013). In general, the findings of this study have led us to believe that hybrids with parent species known for shrubby, rhizamatous growth (e.g., *Q. gambelii* Nutt. and *Q. fusiformis* Small), are more likely to produce rooted shoots using this method.

Our stool bed layering method has been successful in producing rooted shoots (clones) from a large number of our oak hybrids, but some of our genotypes have yet to produce a single rooted shoot. Out of the 345 unique genotypes originally planted out as stock plants, only 235 of them produced rooted shoots at any point during the four years. For those that have been propagated successfully, the percentage of rooted shoots from each stock plant has ranged from approximately 10-70%. The highest number of rooted shoots produced by a stock plant in a year has been 10, but this is an exception, as 3-5 rooted shoots is much more typical.

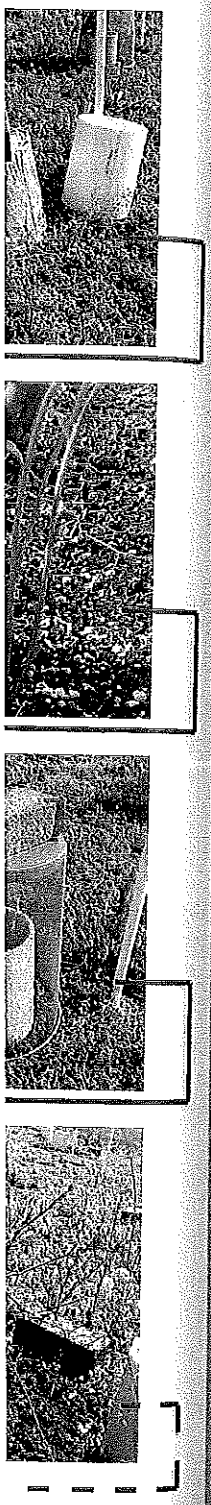
Figure 1

Cloning v

Our stoo
been variab
successfully
3-5 rooted s
limited our
hybrid oak s
of propagat
To addres
methods as
aseptic cond
we are exp
up (dividin
stool bed la
we can start
double the n
approximate
layering me
year, and it
While tis
developed p
Major facto
members of
plant where
course of th
Within th
hybrid oak g
(2009). This
Q. alba L., (
we have bee
using this m
more genoty
experiments
allow us to c
The challe
taxon, differ

Figure 1/ Field
grown for one
cut back to a 3
container used
the new shoots
medium. 6/ Th
becomes acclin
and the mediu
removed, and t
entire procedu

Cloning via tissue culture



Our stool bed layering technique has certainly proven effective, but success rates have been variable between different oak hybrid genotypes. For the plants that have been successfully propagated using this method, the slow rate of multiplication (generally 3-5 rooted shoots per stock plant per year), does not seem commercially viable, and has limited our ability to set up trialing experiments. In order to effectively introduce our hybrid oak selections into the horticultural trade as named cultivars, alternative methods of propagation are required to generate large quantities of clonal plant material.

To address this challenge, we are currently exploring tissue culture (micropropagation) methods as an alternative method. Micropropagation involves propagating plants in aseptic conditions in a laboratory rather than in a field or greenhouse. The main method we are exploring is known as axillary shoot culture. It involves alternatively cutting up (dividing) and growing out shoot segments. This method has advantages over our stool bed layering technique, as it is a continuous, year-round process – meaning that we can start with a single shoot ~1 cm long, and every six weeks we are able to at least double the number of shoots. This makes it possible for us to go from a single shoot to approximately 600 rooted plantlets in a single year. This is far quicker than our stool bed layering method, where a stock plant may produce at most 10 rooted shoots in a single year, and it takes three years to get a stock plant established.

While tissue culture of oaks is by no means common, a handful of researchers have developed protocols that have proven successful with a small number of *Quercus* species. Major factors that have been shown to influence successful initiation of oaks and other members of Fagaceae into tissue culture include: age of stock plants; location on stock plant where explant material is taken; phenological development of the plant over the course of the growing season; and specific genotypes.

Within the last year (2015), we have been exploring propagating our most promising hybrid oak genotypes using an axillary shoot culture method described by Vieitez et al. (2009). This method has already been shown as a way to successfully micropropagate *Q. alba* L., *Q. rubra* L. and *Q. bicolor*. It has proven useful to us over the last year, as we have been able to get six of our hybrid genotypes successfully going in tissue culture using this method. We are currently multiplying these cultures, and plan to initiate more genotypes in the upcoming year. As our numbers increase, they will be used in experiments designed to aid us in our pursuit of an optimized tissue culture protocol, and allow us to conduct further trialing of our selected oak hybrids.

The challenge of genotype specificity is still a major hurdle, meaning that within a given taxon, different genotypes can show varying affinities to induction into tissue culture. It

Figure 1/ Field layering propagation procedure for *Quercus* spp. 0/ The oaks are planted in a field and grown for one to two years for future use as stock plants. 1/ In spring, while the plant is dormant, it is cut back to a 3 cm stump. 2/ When the buds begin to swell, the plant is covered for etiolation. 3/ The container used for etiolation is removed 1 week later. 4/ At this time, IBA is painted onto the bases of the new shoots. 5/ A bottomless container is then placed over the stock plant and filled with a soilless medium. 6/ The plant is then covered with a metal mesh trashcan to temporarily shade the plant as it becomes acclimated to full sun. 7/ After the trashcan is removed, the plant is allowed to grow all season and the medium in the bottomless pot is kept moist. 8/ The following spring, the pot and medium are removed, and the shoots – which hopefully have rooted – are then harvested from the stock plant. The entire procedure can be repeated the following year.

is hypothesized that the developmental stage of plant growth at which explant material is harvested in a given growing season plays a significant role in establishment success. Our research hopes to identify these developmental stages through anatomic observation. We will also focus on optimizing the initiation of the tissue culture process. One of the primary goals of our current research is to develop novel methods that can establish a greater diversity of oak genotypes into tissue culture using a simplified method.

Photographers. Title page: Nina Bassuk (*Q. macrocarpa* × *turbinella*). Photos 0-8: Nina Bassuk and Bryan Denig.

Works cited

Amissah, J., and N.L. Bassuk. 2009. Cutting Back Stock Plants Promotes Adventitious Rooting of Stems of *Quercus bicolor* and *Quercus macrocarpa*. *J. Environ. Hort.*, 27(3): 159-165.

Denig, B.R., P.F. Macrae, X. Gao, and N.L. Bassuk. 2013. Clonal propagation of oak hybrids using a modified layering technique. *Proc. Intl. Plant Prop. Soc.* 63: 253-262.

Denig, B.R., P.F. Macrae, X. Gao, and N.L. Bassuk, 2014. Screening Oak Hybrids for Tolerance to Alkaline Soils. *Journal of Environmental Horticulture* 32: 71-76.

Dirr, M.A. 2010. In Praise of Noble Trees: ASLA Lecture September 12-13. http://www.asla.org/uploadedFiles/CMS/Meetings_and_Events/2010_Annual_Meeting_Handouts/Sun-A3%20In%20Praise%20of%20Noble%20Trees%20(1).pdf

Kriebel, H.B.1993. Intraspecific variation of growth and adaptive traits in North American oak species. Annales des Sciences Forestières 50:153s-165s.

Podaras, P., and E.C. Wells. 2008. Building a Better Oak. *American Nurseryman* 207(8).

Steiner, K.C. 1980. Developing Tree Varieties for Urban Soil Stresses. *Alliance (METRIA) Proc.* 3: 57-69.

Vieitez, A.M., E. Corredoira, A. Ballester, F. Muñoz, J. Durán, and M. Ibarra. 2009. In vitro regeneration of the important North American oak species *Quercus alba*, *Quercus bicolor* and *Quercus rubra*. *Plant Cell, Tissue and Organ Culture (PCTOC)* 98(2): 135-145.

Eating
Far

"In archaec
absence." To n
the archaeolog
importance in
in various diff
significance of
to other nuts, a

To these "a
"incipient agri
between hunte
state desperate
agricultural-ba

This review
the new approa
different story

Keywords: N
memory, Prot