# Screening Oak Hybrids for Tolerance to Alkaline Soils[1]

**Bryan R. Denig, Patrick F. Macrae Jr., Xian Gao, and Nina L. Bassuk[2]**

## Abstract

This study evaluated a diverse range of oak (*Quercus*) hybrids for tolerance to alkaline soils, which is a common site condition in urban landscapes that often limits the growth and longevity of many tree species. Different oak hybrids display varying severities of iron-deficiency induced leaf chlorosis when grown in a highly alkaline medium. Severity of leaf chlorosis was found to vary between different maternal parent species, with the results suggesting that hybrids with the maternal parents *Q. macrocarpa* (bur oak), possibly *Q. muehlenbergii* (chinkapin oak), and *Q.* 'Ooti' (ooti oak), are more likely to maintain healthy green leaf color when growing in a highly alkaline medium. These findings suggest that breeders interested in developing oak hybrids that are both cold-hardy and tolerant of alkaline soils should utilize these species in their crosses, and avoid *Q. bicolor* (swamp white oak), hybrids of which were generally found to be intolerant of alkaline soil. This study is one phase of a long-term project underway at Cornell University's Urban Horticulture Institute to select superior urban-tolerant cultivars of oak hybrids for future introduction into the horticulture industry.

**Index words:** oaks, *Quercus*, urban trees, urban soils, iron deficiency, chlorosis, hybrid.

**Species used in this study:** This study used diverse oak hybrids resulting from crosses involving the following species: ooti oak [*Quercus* 'Ooti' (provisionally accepted; purportedly a cultivar of *Q. robur* L. × *Q. macrocarpa* Michx.× *Q. muehlenbergii* Engelm)]; (*Quercus gambelii* Nutt. × *Q. macrocarpa* Michx); (*Quercus muehlenbergii* Engelm. × *Q. robur* L.); Compton's oak (*Quercus* × *comptoniae* Sarg.); wavy leaf oak (*Quercus* × *undulata* Torr.); Regal Prince® oak (*Quercus* × *warei* T. L. Green & W. J. Hess 'Long'); (*Quercus affinis* Scheidw.); oriental white oak (*Quercus aliena* Blume); bluff oak (*Quercus austrina* Small); swamp white oak (*Quercus bicolor* Willd.); daimyo oak (*Quercus dentata* Thunb.); (*Quercus fabri* Hance); Lusitanian oak (*Quercus fruticosa* Brot.); Texas live oak (*Quercus fusiformis* Small); Gambel oak (*Quercus gambelii* Nutt.); sand live oak (*Quercus geminata* Small); Japanese blue oak (*Quercus glauca* Thunb.); slender oak (*Quercus graciliformis* C. H. Mull.); Lebanon oak (*Quercus libani* G. Olivier); overcup oak (*Quercus lyrata* Walter); caucasian oak (*Quercus macranthera* Fisch. & C. A. Mey. ex Hohen.); bur oak (*Quercus macrocarpa* Michx.); swamp chestnut oak (*Quercus michauxii* Nutt.); dwarf live oak (*Quercus minima* (Sarg.) Small); Mizunara oak (*Quercus mongolica var. grosserata* (Blume) Rehder & E. H. Wilson); chinkapin oak (*Quercus muehlenbergii* Engelm.); Chinese evergreen oak (*Quercus myrsinifolia* Blume); Ubame oak (*Quercus phillyreoides* A. Gray); Mexican white oak (*Quercus polymorpha* Schltdl. & Cham.); dwarf chinkapin oak (*Quercus prinoides* Willd.); English oak (*Quercus robur* L.); net-leaved oak (*Quercus rugosa* Née); (*Quercus spinosa* David); Sonoran scrub oak (*Quercus turbinella* Greene); sandpaper oak (*Quercus vaseyana* Buckley). (Trehane 2007 onwards.)

### Significance to the Horticulture Industry

Alkaline soil is a common site condition in urban areas that restricts the use of several tree species including many oaks. A long-term objective at Cornell's Urban Horticulture Institute is to select superior urban-tolerant cultivars of oak hybrids for future introduction into the nursery industry. By using hybrids, it is thought that desirable characteristics from tender species can be combined with cold-hardiness, and cultivars can be selected and introduced in order to greatly increase the palette of oaks suitable for planting in temperate urban landscapes. This experiment involved screening oak hybrids for tolerance to alkaline soils, and the results demonstrate that different oak hybrids display varying severities of iron-deficiency induced leaf chlorosis when grown in a highly alkaline medium. The maternal parent species *Q.* 'Ooti', *Q. macrocarpa*, and *Q. muehlenbergii* were associated with hybrids that are especially tolerant of alkaline soils. Tree breeders interested in developing oak hybrids that are both cold-hardy and tolerant to alkaline soils should consider utilizing these species in their crosses.

[1]Received for publication January 27, 2014; in revised form June 5, 2014. Preliminary research supporting this project was partially funded by by **The Horticulture Research Institute, 1200 G Street NW, Suite 800, Washington DC 20005**.

[2]Graduate Student/Research Technician and corresponding author (brd45@cornell.edu), Graduate Student/Research Technician, Graduate Student Alumnae, and Professor. Urban Horticulture Institute, Department of Horticulture, Cornell University, Ithaca, NY 14853.

### Introduction

A major obstacle to using certain temperate oaks (*Quercus* spp.) found in the northeastern United States is their intolerance of the alkaline soils inherent to many urban landscapes. Many native oak species suitable for planting in the northeastern United States display iron deficiency symptoms, such as interveinal chlorosis, poor root formation, and growth retardation when grown in alkaline soils. A plant's tolerance of alkaline soils depends on its ability to take up nutrients that are less soluble at a higher soil pH. Essential nutrients, such as iron, manganese, copper, and zinc, although possibly abundant in the rhizosphere, become significantly less available in higher pH soils. Iron is especially important as it plays an irreplaceable role in functional processes such as photosynthesis and respiration. Planting sites in urban landscapes commonly contain alkaline soils resulting from the release of carbonate from construction materials, and this site characteristic can limit the palette of tree species that can be grown successfully (Jim 1997).

One method for increasing the usefulness of certain trees in urban environments involves taking advantage of intraspecific variation within the population of the species of interest, and selecting for cultivars tolerant of urban stresses such as alkaline soils (Steiner 1980). This is a worthwhile approach for oaks since 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). One example of selecting for urban tolerant traits in oaks is seen in the work done at Penn State University to select a chlorosis-resistant pin oak (*Q. palustris*) (Berrand

  

1979). While this species may be one of the most popular street tree species, the pin oak, along with certain other street tree species, will often display symptoms of an iron deficiency, such as chlorotic leaves and poor growth, when grown in alkaline soils (Wallace and Wallace 1986). Work at Penn State University demonstrated genetic variability of chlorosis-resistance in pin oaks, finding that plants from populations in Indiana, Illinois, and Missouri were consistently among the most resistant to iron-deficiency chlorosis (Berrand and Steiner 1980).

Rather than making selections from genetically-varied native populations to obtain oaks tolerant of urban stresses, another strategy for expanding the palette of oaks suitable for urban landscapes involves 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 not sharing the same geographic range can increase the chance of finding alkaline soil tolerant individuals. Hybrids could potentially combine tolerance to alkaline soils 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 hybrid oak selections become available (Dirr 2010; Schmidt n.d.).

The objective of this study was to screen a number of developed hybrid oaks for tolerance to alkaline soils. The hybrids used have proven winter hardy over the last five years in Ithaca, New York, and represent crosses of over 40 diverse parent species, most of which belong to the white oak taxonomic group.

## Materials and Methods

This study utilized clones of oak hybrids developed by Peter Podaras at Cornell University in Ithaca, NY (Podaras and 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 in Ithaca, NY, with pollen from 36 species native throughout North America, Europe, and Asia. Because the female flowers of an oak tree mature before the male catkins do, controlled pollinations were possible with careful timing. Additionally, after acorns from these crosses were grown, plants were monitored for one to two years to confirm that their leaves displayed intermediate characteristics when compared to the leaves of their parents (Podaras and Wells 2008). Individual acorns were germinated in containers and grown for two to four years in a greenhouse and/or a polyhouse before planting outdoors. During the spring of 2008, 345 unique hybrid oak genotypes, the results of the controlled crosses done in 2004, 2005, and 2006, were planted in the research fields at Cornell University in USDA hardiness zone 5b (USDA 2012) in an Arkport sandy loam with a pH of 6.2 for future use as stock plants. Starting in spring 2009, and repeated yearly through 2012, these field-grown stock plants were asexually propagated each year using the oak stoolbed layering propagation protocol developed by Amissah and Bassuk (2009) (Denig et al. 2013).

The plants used in this study were the result of asexually propagating the hybrid oak stock plants during the 2011 season. The rooted shoots were harvested from each stock plant in March 2012 and potted in #1 containers. A 1:1 (v/v) peat:perlite soilless mix with dolomitic limestone added at the rates of 0.593 $kg \cdot m^{-3}$ (1 $lb \cdot yd^{-3}$) and 11.866 $kg \cdot m^{-3}$ (20 $lb \cdot yd^{-3}$) to adjust the pH of the medium to 6.0 or 8.0 respectively, was used. Replicate clones of the same genotype (a result of multiple rooted shoots being harvested from the same stock plant) were divided to include both acid and alkaline replicates if possible, with preference given to using the alkaline medium when potting the plants. All harvested shoots showing root growth were planted in #1 containers no matter how small the root system. As expected, several of the oaks died in the weeks following planting. This die-off, along with the unequal starting numbers, resulted in a varied number of replicates between genotypes. Out of the 345 genotypes growing in the field and being used as stock plants, some were not represented in the greenhouse experiment, others only had one replicate, and still other genotypes, the ones whose layers readily rooted that year, had a large number of replicates (Table 1).

*First season in greenhouse.* After potting, the oaks were placed in a greenhouse where average conditions consisted of 23.9C (75F) day and 18.3C (65F) night temperatures, with plants being watered as needed. The plants were fertilized with 150 ppm NPK (21-5-20) every 2 weeks, and sprayed with pesticides as needed to control greenhouse pests such as mildew and aphids.

In addition to regular fertilization, 3 g (0.106 oz) of a granular micronutrient product (Micromax, Everris NA, Inc., Dublin, OH) was added onto the surface of the growing medium in each pot. This was done at the beginning of the season to insure that micronutrients, especially iron (Fe), were present in the medium. While some of the potted oaks readily developed new shoots after breaking dormancy, others were much slower in developing new growth. A gibberellic acid (GA) solution of 500 ppm $GA_{4+7}$ in 5% aqueous ethanol was dabbed on the dormant terminal buds of the trees that did not grow every 4 days until the plant displayed a new lengthening shoot, using a method adapted from a prior study (Bond 1998).

For each plant, a newly-developed shoot was identified. From this shoot, 3 newly-developed, but fully expanded, adjacent leaves were chosen. A mark was made on each with a permanent marker so that consecutive readings could be taken from the same leaves to track leaf chlorosis in plants over time. A SPAD-502 chlorophyll meter (Konica Minolta, Inc. Tokyo, Japan) was used. This device produces readings on an arbitrary quantified scale that correlate highly with actual chlorophyll concentration (Bond 1998). A SPAD (Special Products Analysis Division, Konica Minolta, Inc.) value of less than 20 corresponds with a very chlorotic cream to white colored leaf, and leaves with SPAD values over 30 are not visibly chlorotic and show a healthy green color. Leaves with SPAD values between 45 and 55 are especially dark green in color. When using the SPAD meter, care was taken to avoid the leaf midrib and veins, which might result in an inaccurate reading. SPAD readings were taken on the 3 selected leaves, and these were then averaged together. The average value of the three leaves was recorded for each plant. Additional SPAD readings were taken for each plant

**Table 1. Summary of oak crosses used in the greenhouse alkaline soil tolerance screening experiment.**

| Maternal parent species | Paternal parent species | Number of genotypes used | Number of plants used |
|---|---|---|---|
| *Q. bicolor* | open pollinated | 4 | 16 |
| *Q. bicolor* | *Q. affinis* | 1 | 3 |
| *Q. bicolor* | *Q. aliena* | 11 | 25 |
| *Q. bicolor* | *Q. austrina* | 1 | 2 |
| *Q. bicolor* | *Q. dentata* | 3 | 8 |
| *Q. bicolor* | *Q. fabri* | 7 | 16 |
| *Q. bicolor* | *Q. fruticosa* | 4 | 10 |
| *Q. bicolor* | *Q. geminata* | 1 | 4 |
| *Q. bicolor* | *Q. glauca* | 1 | 2 |
| *Q. bicolor* | *Q. graciliformis* | 4 | 12 |
| *Q. bicolor* | *Q. libani* | 3 | 4 |
| *Q. bicolor* | *Q. lyrata* | 1 | 3 |
| *Q. bicolor* | *Q. macranthera* | 1 | 1 |
| *Q. bicolor* | *Q. minima* | 3 | 6 |
| *Q. bicolor* | *Q. mongolica grosserata* | 2 | 5 |
| *Q. bicolor* | *Q. muehlenbergii* | 6 | 17 |
| *Q. bicolor* | *Q. muehlenbergii* × *Q. robur* | 3 | 16 |
| *Q. bicolor* | *Q. myrsinifolia* | 10 | 31 |
| *Q. bicolor* | *Q. phillyreoides* | 1 | 1 |
| *Q. bicolor* | *Q. polymorpha* | 1 | 1 |
| *Q. bicolor* | *Q. robur* | 5 | 10 |
| *Q. bicolor* | *Q. rugosa* | 7 | 22 |
| *Q. bicolor* | *Q.* sp. | 5 | 8 |
| *Q. bicolor* | *Q. turbinella* | 3 | 8 |
| *Q. bicolor* | *Q. vaseyana* | 4 | 12 |
| *Q. gambelii* × *Q. macrocarpa* | *Q. lyrata* | 2 | 7 |
| *Q. gambelii* × *Q. macrocarpa* | *Q.* × *comptoniae* | 1 | 1 |
| *Q. macrocarpa* | open pollinated | 5 | 14 |
| *Q. macrocarpa* | *Q. gambelii* | 1 | 5 |
| *Q. macrocarpa* | *Q. lyrata* | 1 | 1 |
| *Q. macrocarpa* | *Q. michauxii* | 2 | 3 |
| *Q. macrocarpa* | *Q. minima* | 1 | 3 |
| *Q. macrocarpa* | *Q. prinoides* | 2 | 4 |
| *Q. macrocarpa* | *Q. turbinella* | 2 | 2 |
| *Q. macrocarpa* | *Q.* × *undulata* | 2 | 7 |
| *Q. macrocarpa* | *Q.* × *comptoniae* | 1 | 5 |
| *Q. muehlenbergii* | open pollinated | 1 | 1 |
| *Q. muehlenbergii* | *Q. prinoides* | 1 | 1 |
| *Q.* 'Ooti' | *Q. fusiformis* | 1 | 3 |
| *Q.* × *warei* 'Long' | open pollinated | 5 | 18 |
| *Q.* × *warei* 'Long' | *Q.* × *comptoniae* | 15 | 46 |

1 month and 2 months after the initial reading. Each time, the average value of the same 3 leaves was recorded. By recording average SPAD readings on the same leaves for a period of 3 months, the data were expected to provide a timeline of leaf chlorophyll development over the growing season for each plant.

Due to the poor new shoot development of some plants, and the use of GA, the plants were asynchronous in the growth of new shoots and the development of leaves. In order for all plants to be at the same leaf stage during SPAD readings, the oaks were divided into 3 groups. Due to the varying phenology of the plants, SPAD readings were done for an early group (July 10, August 7, September 4), middle group (July 24, August 20, September 18), and late group (August 7, September 4, October 2). The groups were formed based entirely on the phenology of each plant, and which hybrid parent species were involved in each cross did not appear to correlate with which plants composed each group. At the end of the season, after all SPAD data were collected, the oaks were placed in a cooler set to 4.4C (40F) for a period of induced dormancy. They entered the cooler on October 3, 2012, and were removed on February 4, 2013 and placed into a greenhouse in order to collect another season's worth of data.

*Second season in greenhouse.* Average greenhouse conditions consisted of 23.9C (75F) day and 18.3C (65F) night temperatures, plants were watered as needed, fertilized with 300 ppm NPK (21-5-20) twice a week, and sprayed with pesticides as needed to control mildew and aphids. Supplemental overhead lighting with a 16-hour photoperiod was used, as winter days in Ithaca, NY, provide limited sunlight. A 3 g (0.106 oz) amount of Micromax was given to each plant. By early March, every oak was actively growing. No plants were lost between the first and second seasons in the greenhouse.

Unlike the first season, all of the trees possessed buds that readily expanded into new shoots, so application of GA was not needed. Oaks usually demonstrate episodic shoot growth, and they often complete two flushes of growth during a typical growing season. For each plant, 2 SPAD readings were taken on the first flush of growth, and 2 more were taken on the second flush of growth. Unlike the first season in the greenhouse, the oaks were acceptably synchronized in their shoot and leaf development, and SPAD readings for all the oaks were done on the same days. SPAD readings were taken twice on the first flush of growth (March 19 and April 15) and twice on the second flush of growth (May 24 and June 20). For each plant, 3 leaves were selected, and the average

SPAD was recorded. These same leaves were evaluated a month later. For the final SPAD readings, 3 leaves were selected from the second flush of growth, the average SPAD was recorded, and these were evaluated a month later. On June 15, before the final reading, 3 g (0.106 oz) Micromax was again added to each pot, to insure the presence of iron and other micronutrients.

*pH adjustments.* Using a pour-thru pH testing method (Thomas 2010), random testing of pots suggested that the pH values of the medium were shifting away from the original design of pH 6.0 and pH 8.0. The pH decreased over time in the alkaline growing medium, while it increased in the acidic medium. In an effort to reverse this change, the plants were all given a pH-modifying drench. Plants in the acid medium treatment received sulfuric acid at a rate of 0.30 g sulfuric acid·$L^{-1}$ (4 oz·100 $gal^{-1}$) on March 6, 2013. Plants in the alkaline treatment received a potassium bicarbonate drench at a rate of 1.20 g $KHCO_3 \cdot L^{-1}$ (1 lb·100 $gal^{-1}$) on March 15, 2013. Drenches were administered with a Dosatron, a water-powered proportional dosing device manufactured by Doastron International, Inc. (Clearwater, FL). Testing of 10 pots from the alkaline treatment and 10 pots from the acid treatment before and after administering the drenches demonstrated the mitigating effects of the drenches. The mean pH for the tested acid treatment pots decreased from 6.56 to 6.23, and the mean pH for the tested alkaline treatment pots increased from 7.5 to 7.7. While these new values differed from the experiment intent of having treatments at pH 6.0 and pH 8.0, they were acidic and basic enough for the purposes of the study.

*Testing for iron deficiency.* After the completion of the second season of SPAD measurements, we checked whether the leaf chlorosis and stunted growth observed were due to an iron deficiency. For this test, 10 of the most chlorotic plants growing in the alkaline medium in the greenhouse were selected, as well as 10 replicates of these genotypes which were growing in the acid medium and therefore not displaying leaf chlorosis. Sprint® 138, a 6% fully chelated ethylenediamine-N,N'-bis(2-hydroxyphenylacetic acid) (EDDHA) iron product (manufactured by Becker Underwood Inc. Ames, IA) that prevents iron from binding with other compounds in the soil or medium, even in high pH conditions, was used to supply each plant with readily-available iron. After a thorough watering, each of the 20 plants received 0.85 g (0.03 oz) of Sprint® 138 dissolved in approximately 500 mL (~16.9 fl oz) water. After this drained from the pots, plants received an additional 0.85 (0.03 oz) grams of Sprint® 138 dissolved in ~500 mL (~16.9 fl oz) water. SPAD measurements were taken on all 20 plants before the addition of the chelated iron, and again 3 weeks later, after the plants had sufficient time to absorb the chelated iron.

*Statistical analysis.* JMP Pro 10.0.2 (SAS Institute 2012) was used for statistical analysis of SPAD readings which were grouped by date (before or after the chelated iron drench), as well as pH class (pH 6.0 or pH 8.0). The model included an interaction between these two variables, and tree genotype as well as individual plant identity were defined as random effects. Least square means was used to make comparisons between the SPAD readings for the different pH classes before and after the addition of Sprint® 138. Least square means comparisons were also done within each pH class. The Tukey-Kramer HSD test was used to compare differences of SPAD value.

Statistical analysis of the leaf chlorosis data was done using SAS 9.3 statistical software (SAS Institute Inc. 2011), and the unit for analysis was a single SPAD reading. It was observed that some leaves, while fully developed in size, would temporarily display a reddish tint (an ornamental characteristic observed in some of the hybrids) or some chlorosis while young, yet subsequently turn green. Because the SPAD measurements taken while leaves are displaying these temporary characteristics overemphasize the effects of the alkaline medium on leaf chlorophyll concentrations, only the final SPAD measurements for each of the three growth flushes were used for statistical analysis. 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 were pooled by maternal parent species and pH class for analysis. The effect of the maternal parent species on the SPAD value for each of the pH classes was studied.

In order to study the effect of the maternal parent species and pH class on SPAD reading, a generalized linear mixed model was employed. The combination of parents (hybrid species), genotype, and individual tree identity were controlled for as random effects. Least squares means was used to estimate the SPAD reading based on the maternal parent and pH class. Because statistical analyses with multiple comparisons can result in artificially low *P* values, a Bonferonni correction for multiple comparisons was done to adjust *P* values and thereby increase the significance threshold. The Tukey-Kramer method was used to compare maternal parent least square means with differences being considered significant when $P < 0.05$.

**Results and Discussion**

*Testing for iron deficiency.* Before the addition of chelated iron, mean SPAD values were found to vary significantly between the healthy green-leaved pH 6.0 test group and the highly chlorotic pH 8.0 test group, with a difference of 33.97 SPAD points between the two groups (Fig. 1). The mean SPAD values for the readings taken 3 weeks after the plants received the chelated iron drench were also found to vary significantly, but the differences were smaller than before, at 15.59 SPAD points. This indicates that the leaf chlorophyll concentration of the pH 8.0 plants increased considerably. As expected, mean SPAD values between pH 8.0 plants from before and after the addition of chelated iron were significantly different, with an 18.26 difference. By comparison, readings for pH 6.0 plants did not significantly change after the addition of chelated iron.

*Testing for tolerance to alkaline soils.* Within both the pH class and maternal parent effects, the mean SPAD values were found to vary significantly (Fig. 2). The mean SPAD values were not found to vary significantly between maternal parent species within the pH 6.0 class, but they did vary significantly within the pH 8.0 class. Within each of the maternal parents, the mean SPAD values were found to vary significantly between the two pH classes, with the exceptions of *Q. muehlenbergii* and *Q.* 'Ooti' (Table 2), suggesting that the hybrids with these two species as maternal parents perform equally well, at least in terms of leaf greenness, growing in high or low pH conditions. The pH 8.0 SPAD mean values

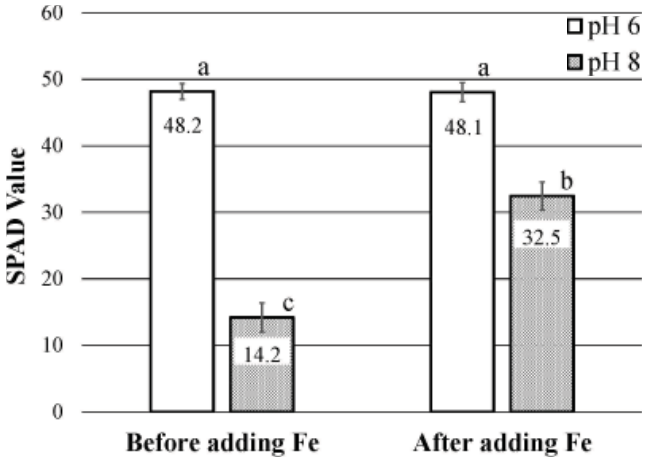


**Fig. 1. SPAD value estimates before and after the addition of chelated iron, averaged across oak species, in the greenhouse experiment. Different letters indicate highly significant differences (*P* < 0.0001, Tukey-Kramer HSD test). Error bars represent calculated standard error. A SPAD (Special Products Analysis Division, Konica Minolta, Inc.) value of less than 20 corresponds with a very chlorotic cream to white colored leaf, and leaves with SPAD values over 30 are not visibly chlorotic and show a healthy green color. Leaves with SPAD values between 45 and 55 are especially dark green in color.**

were highest in the hybrids with the maternal parents *Q.* 'Ooti' (40.58), *Q. macrocarpa* (35.65), and *Q. muehlenbergii* (31.07). The maternal parent with the lowest pH 8.0 SPAD mean value was *Q. bicolor* (20.94), indicating that hybrids that possess this maternal parent tend to be intolerant of alkaline soils (Fig. 2).

Unfortunately, the maternal parents *Q. muehlenbergii* and *Q.* 'Ooti' both had limited replication in the experiment. *Q. muehlenbergii* as a maternal parent was only represented

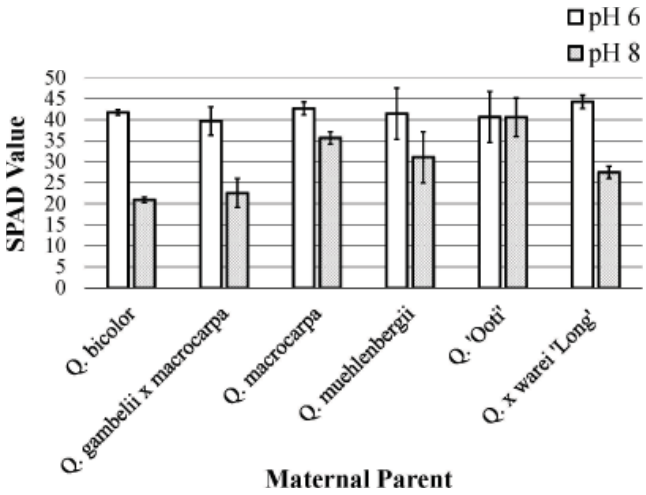


**Fig. 2. SPAD value estimates based on pH class and maternal parent for oak hybrids grown in a greenhouse. Error bars represent calculated standard error. A SPAD (Special Products Analysis Division, Konica Minolta, Inc.) value of less than 20 corresponds with a very chlorotic cream to white colored leaf, and leaves with SPAD values over 30 are not visibly chlorotic and show a healthy green color. Leaves with SPAD values between 45 and 55 are especially dark green in color.**

**Table 2. Statistics of significant differences between pH classes of 6.0 and 8.0 within maternal parents for oak hybrids grown in a greenhouse trial.**

| Maternal parent | *F* value | Original p-value | Adjusted p-value |
|---|---|---|---|
| *Q. bicolor* | 757.48 | <0.0001 | <0.0036 |
| *Q. gambelii* × *Q. macrocarpa* | 17.88 | <0.0001 | <0.0036 |
| *Q. macrocarpa* | 15.18 | 0.0001 | 0.0036 |
| *Q. muehlenbergii* | 1.46 | 0.2287 | 8.2332 |
| Q. 'Ooti' | 0 | 0.9942 | 35.791 |
| *Q.* × *warei* 'Long' | 126.99 | <0.0001 | <0.0036 |

by 2 plants, while only 3 plants had *Q.* 'Ooti' as a maternal parent. This lack of replication makes it difficult to make statistically-sound conclusions about these species, but observation does suggest that the hybrids with these maternal parents are indeed tolerant of alkaline soils.

The pH 8.0 *Q. muehlenbergii* SPAD value is not significantly different from the pH 8.0 SPAD values of any of the other maternal parent species, and there was a significant difference between the pH 8.0 *Q. bicolor* SPAD value and the pH 8.0 values of Q. *macrocarpa*, *Q.* 'Ooti', and *Q.* × *warei* 'Long' (Fig. 3). The limited number of plants with *Q. gambelii* × *Q. macrocarpa*, *Q. muehlenbergii*, and *Q.* 'Ooti' as maternal parents resulted in high standard error values for these species.

The relatively high pH 8.0 SPAD mean values observed in hybrids with the maternal parents *Q. macrocarpa*, *Q. muehlenbergii*, and *Q.* 'Ooti', are likely due to the inherent tolerance of alkaline soils associated with these species. *Q. macrocarpa* and *Q. muehlenbergii* are both widely distributed North American species that generally prefer calcareous and limestone soils (Miller and Lamb 1985). *Q.* 'Ooti' is

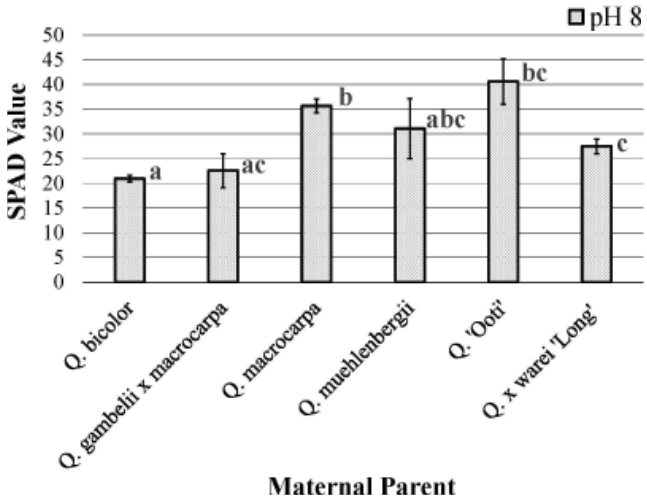


**Fig. 3. SPAD value estimates for pH 8.0 treatment based on maternal parent for oak hybrids grown in a greenhouse. Different letters indicate significant differences among maternal parents in the pH 8.0 treatment. (*P* < 0.05, Tukey-Kramer HSD test). Error bars represent calculated standard error. A SPAD (Special Products Analysis Division, Konica Minolta, Inc.) value of less than 20 corresponds with a very chlorotic cream to white colored leaf, and leaves with SPAD values over 30 are not visibly chlorotic and show a healthy green color. Leaves with SPAD values between 45 and 55 are especially dark green in color.**

a cultivar of difficult to determine origins, supposedly a selection of the complex hybrid *Q. robur* × *Q. macrocarpa* × *Q. muehlenbergii (Pavia Nursery n.d.).* All three of these species are known to be alkaline soil tolerant (Dirr 2009). Additionally, *Q.* 'Ooti' is only represented by a single cross and genotype in this experiment, and the observed alkaline soil tolerance might also be connected to the paternal parent species involved, *Q. fusiformis*, the Texas live oak. This often shrubby, rhizomatous, mostly evergreen species is similar to *Q. virginiana*, the Southern live oak, but more cold-hardy and tolerant of alkaline soils (Grimshaw and Bayton 2009). Because the maternal parent *Q.* 'Ooti' lacks multiple paternal parent species in this experiment, these results cannot be used to infer that *Q.* 'Ooti' as a maternal parent will likely result in offspring hybrid oaks that are tolerant of alkaline soils. What can be deduced is that the only *Q.* 'Ooti' × *Q. fusiformis* genotype in this experiment, which has a supposed pedigree consisting of four oak species known to be tolerant of calcareous and limestone soils, could potentially make a worthwhile introduction into the horticulture trade as a tree that can withstand the highly alkaline soils common in urban landscapes.

As *Q. gambelii* and *Q. macrocarpa* are both species known to be tolerant of alkaline soils (Sternberg and Wilson 2004), hybrids with *Q. gambelii* × *Q. macrocarpa* as a maternal parent might be expected to display alkaline soil tolerance, but this was not observed. This is possibly due to the influence of the pollen parent species crossed with *Q. gambelii* × *Q. macrocarpa*. Interestingly, *Q. bicolor*, the maternal parent species with the greatest number of replicates, unique hybrids, and unique genotypes in this experiment, is associated with a relatively low pH 8.0 SPAD mean value. *Q. bicolor* is known to display severe leaf chlorosis when grown in alkaline soils (Sternberg and Wilson 2004). Although a diverse range of *Q. bicolor* crosses were used in this experiment, the innate alkaline tolerence is unknown for the majority of the 25 pollen parent species. Because of this, it is difficult to conclude if the low pH 8.0 mean SPAD value for *Q. bicolor* is due to the singular or combined effects of the maternal and paternal species involved in each cross. In varying degrees, the difficulties of drawing conclusions about the singular and combined effects of the maternal and paternal parent species on their hybrid offspring holds true throughout this experiment.

The developed *Quercus* hybrids, while they perform similarly in terms of leaf chlorosis when grown in an acid medium, display significant differences when grown in an alkaline medium. While analyzing data that are grouped by maternal parent species might obscure which individual hybrids are indeed tolerant of alkaline soils, observations can be made from the raw data to predict which of the hybrid oak genotypes have the potential to be introduced as alkaline soil tolerant cultivars. Interestingly, observations suggest that half-sibling hybrid genotypes, even though they resulted from the same cross, can vary substantially in their tolerance to alkaline soils.

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 tolerant of alkaline soils. Additionally, the findings will be used to select from the developed hybrids the highly alkaline soil tolerant genotypes for future observation. This study is just one step in the long-term project of Cornell University's Urban Horticulture Institute to select, develop an asexual propagation protocol for, and introduce named cultivars of superior urban-tolerant oak hybrids.

## Literature Cited


Amissah, J.N. and N.L. Bassuk. 2009. Cutting back stock plants promotes adventitious rooting of stems of *Quercus bicolor* and *Quercus macrocarpa*. J. Environ. Hort. 27:159–165.

Berrang, P.C. 1979. Intraspecific variation in resistance to iron chlorosis in pin oak. M.S. Thesis. Pennsylvania State University, University Park, PA. p. 1–4.

Berrang, P.C. and K.C. Steiner. 1980. Resistance of pin oak progenies to iron chlorosis. J. Amer. Soc. Hort. Sci. 105:519–522.

Bond, G.A. 1998. Screening oak species for iron efficiency. M.S. Thesis. Cornell University, Ithaca, NY. 37 pp.

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. 63:1.

Dirr, M.A. 2009. Manual of Woody Landscape Plants: Their Identification, Ornamental Characteristics, Culture, Propagation and Uses. Stipes Pub., Champaign, IL. p. 925–926, 928–929, 937–940.

Dirr, M.A. 2010. In Praise of Noble Trees: ASLA Lecture. http://www.asla.org/uploadedFiles/CMS/Meetings_and_Events/2010_Annual_Meeting_Handouts/Sun-A3%20In%20Praise%20of%20Noble%20Trees%20(I).pdf. Accessed May 28, 2014.

Grimshaw, J. and R. Bayton. 2009. New Trees: Recent Introductions to Cultivation. Royal Botanic Gardens, Kew, Richmond, Surrey. 723 pp.

J. Frank Schmidt & Son Co. n.d. Our Introductions. http://www.jfschmidt.com/introductions/index.html. Accessed May 28, 2014.

Jim, C.Y. 1997. Urban soil characteristics and limitations for landscape planting in Hong Kong. Landscape Urban Plan. 40:235.

Kriebel, H.B. 1993. Intraspecific variation of growth and adaptive traits in North American oak species. Ann. Sci. Forest. 50(supplement):153s–165s.

Miller, H.A. and S.H. Lamb. 1985. Oaks of North America. Naturegraph Publishers, Happy Camp, CA. p. 153–158, 188–191.

Pavia Nursery. n.d. Catalogue: Q. http://www.pavia.be/catalogue/show/q. Accessed May 28, 2014.

Podaras, P. and E.C. Wells. 2008. Building a better oak. Am. Nur. 207:22–24.

Steiner, K.C. 1980. Developing tree varieties for urban soil stresses. Metro. Tree Improvement Alliance (METRIA) Proc. 3:57–69.

Sternberg, G. and J.W. Wilson. 2004. Native Trees for North American Landscapes: From the Atlantic to the Rockies. Timber Press, Portland, OR. p. 368–380.

Thomas, J.N. 2010. Identifying rhododendrons tolerant of alkaline soils: Is iron the limiting nutrient in high pH soils? Master of Professional Studies Project Report. Cornell University, Ithaca, NY. p. 9–10.

Trehane, P. 2007 onwards. The Oak Names Checklist. http://www.oaknames.org. Accessed May 2013.

USDA Plant Hardiness Zone Map. 2012. Agricultural Research Service, U.S. Department of Agriculture. http://planthardiness.ars.usda.gov. Accessed May 28, 2014.

Wallace, A. and G.A. Wallace. 1986. Ornamental plants most likely to be killed by iron deficiency and some control measures. J. Plant Nutr. 9:1009–1014.