Assessing the Tolerance of Three Species of *Quercus* L. and Iowa Grown *Betula nigra* L. Provenances to Foliar Chlorosis in Elevated pH Substrate

by

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B.S., Kansas State University, 2015

A THESIS

submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Department of Horticulture and Natural Resources
College of Agriculture

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2018

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Abstract

Oak trees (*Quercus* L.) and river birch (*Betula nigra* L.) are two horticulturally significant crops widely used in landscapes but notorious for developing iron (Fe) induced interveinal foliar chlorosis (IFC) in alkaline soils. Variation in IFC has been observed between species of oak and provenances of river birch suggesting that species and provenances endemic to alkaline soils do not always display this chlorosis. Limited studies investigating the effect of elevated pH on oak and river birch have been conducted. More environmentally tolerant and aesthetically pleasing selections could be used if they are first screened to determine their adaptability to high pH soils.

Three experiments were conducted to evaluate Texas red oak (*Quercus buckleyi* Nixon and Dorr) and Durand oak [*Quercus sinuata* Walter *var. breviloba* (Torr.) C.H. Mull.] with landscape collections of pin oak (*Quercus palustris* L.) to determine the extent of IFC when grown at elevated pH. When grown in an elevated pH substrate, pin oak was unable to maintain elevated leaf total leaf Fe concentrations, consistently developed IFC, and exhibited low total leaf chlorophyll concentrations compared to non-chlorotic pin oak seedlings in the control pH substrate. Texas red oak and Durand in the elevated substrate did not develop IFC and maintained high leaf chlorophyll concentrations compared to controls; they also sequestered greater amounts of substrate Fe in leaves compared to pin oak in the elevated substrates.

Another crop of ornamental significance and widely planted in the landscape, river birch (*Betula nigra* L.), develops IFC in high pH soils. Two experiments evaluated river open-pollinated (OP) seedlings of Iowa provenances, OP ‘BNMTF, and clones from selected Iowa provenances, ‘BNMTF’, ‘Cully’ in an elevated pH substrate. A seed source from Bearbower Sand Prairie, Buchanan Co., IA (BSP3) had greater leaf chlorophyll than ‘BNMTF’OP, and a

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Acknowledgements

The author acknowledges the constant encouragement and patience from his committee members, Drs. Chad T. Miller and Kim A. Williams of the Department of Horticulture and Natural Resources at Kansas State University and Dr. Jason J. Griffin of the Department of Horticulture and Natural Resources, Kansas State University and Director of the John C. Pair Horticulture Center, Haysville, KS. He thanks them for their unwavering support of his personal and academic developments and research interests. For the support of his studies, he also acknowledges and thanks The Kansas Forest Service and Tim McDonnell the District Community Forester of South Central, Southeast Districts for the KS Forest Service.

Further, he acknowledges Ryan Reitz of Kerr Wildlife Management Area, Hunt, Texas and staff of the Lost Maples State Natural Area, Vanderpool TX from Texas Parks and Wildlife for their assistance with *Q. buckleyi* and *Q. sinuata* var. *breviloba* seed collection. In addition, he thanks Jeffery Carstens and staff of the North Central Region Plant Introduction Station (Agricultural Research Service - United States Department of Agriculture, Ames, Iowa) for obtaining the Iowa native *Betula nigra* sources, and Eric Clark and staff of Greenleaf Nursery Co., Park, Hill, Oklahoma for *B. nigra* 'Cully' used for this research. Furthermore, he thanks Fred Hulme at Everris for the supply of Osmocote® and Micromax® fertilizers.

Finally, he acknowledges and appreciates the insight and invaluable reflection given to this work by Michael Shelton of The John C. Pair Horticulture Center and assistance for data collection from Ethan, Eric, and Evan Copeland, Tyler Blasters, and Tami Myers.

Chapter 1 - Interveinal Foliar Chlorosis of Species of Oak and River Birch

Introduction

Oak trees have been an integral part of American history over the past hundred years, and they will continue to serve as a dominant and picturesque species in the landscape (Sternberg and Wilson, 1995). In 2014, annual oak (*Quercus* L.) sales in the United States (U.S) topped 105.5 million dollars and accounted for 18.8% of all deciduous tree sales in the U.S. (USDA Agriculture Census, 2014). When planted correctly and in the appropriate location, oaks can be the longest living landscape species in North America. For example, live oak (*Quercus virginiana* Small), a signature of the deep south, are some largest and oldest landscape trees, well-defined by their arching branches, tapered trunks, and host for the ornamental Spanish moss (*Tillandsia usneoides* L.). Furthermore, many species live for centuries; a 250-year white oak (*Quercus alba*) stands at the Morton Arboretum (The Morton Arboretum, 2015). In addition to bringing longevity to a landscape, oaks have the capability of sequestering large amounts of carbon over their lifetime, more than any other hardwood species (Brown, n.d.; Jacobs, 2014). Nowak (2013) reported that urban trees throughout the U.S. are sequestering 25.6 million tons of carbon annually, where over 643 million tons has already been sequestered. Along with reducing atmospheric carbon large trees, like oaks, may have the potential to reduce energy costs in the landscape. McPherson (1993) reported a single deciduous landscape tree, approximately 7.6 m tall, could reduce annual-air conditioning costs for homeowners by 8% to 10%. In a different scenario, street trees have been found to reduce urban heat islands by blocking solar radiation from reaching the pavement (McPherson, 2005). Even though oaks are long-lived, sequester large volumes of carbon, reduce energy costs, and provide an aesthetic addition to the landscape,

the foremost concern when planting species of oak should be that of sustainability in the landscape. North America is home to roughly 60 species of oak and their landscape performance depends on the edaphic factors (e.g. soil type, pH, mineral composition, etc.) of the site (Miller and Lamb, 1985). One specific sustainability concern of species of oak in the landscape, is their susceptibility to foliar chlorosis induced by the unavailability of iron (Fe) in elevated pH soils (Figure 1.1).

A better awareness for sustainability in the landscape has been developed since the invasion of Dutch elm disease (*Ophiostoma novo-ulmi* Brasier), emerald ash borer (*Agrilus planipennis* Fairmaire), and sudden oak death [*Phytophthora ramorum* (Werres, De Cock & Man in 't Veld)] (Cappaert et al., 2005; Karnosky, 1979; Kliejunas, 2010) into landscapes. Furthermore, the development of sustainable landscapes can be sought by incorporating greater plant genetic diversity and by using appropriate plant selection (Santamour, 1990). Considering plant selection, trees susceptible to interveinal foliar chlorosis (IFC), caused by a lack of micronutrients, are not sustainable, because, foremost, the impact of IFC on a tree's health is quite substantial (Hauer and Dawson, 1996). If untreated, it is common for tree impacted by IFC to experience reduction in growth followed, by tree decline, and eventual tree mortality. The reduction in growth, due to an Fe deficiency, can be attributed to an inability to produce photosynthetic products and lack of metabolic function to sustain optimal health (Neely, 1976). Plant-induced physiological stressors may develop too. Under Fe deficiency, a lack of photosynthetic activity (PSII) results in the accumulation of reactive oxygen species (ROS) such as superoxide anions and hydrogen peroxide. ROS accumulation leads to programmed cell death (Hellin et al., 1995; Tewari et al., 2013).Also, secondary tree decline can occur, because IFC increases a tree's susceptibility to biotic, abiotic, and physiological stressors. Tubakia leaf spot

(*Actinopelte dryina* Sacc.) can cause premature leaf defoliation in nutritionally deficient oaks in late summer (Olsen, 2017). In addition, marginal necrosis and leaf scorch is prevalent on chlorotic plants under salt, heat, drought, heavy metal stress (Akbari et al., 2013; Emamverdian et al., 2015; Neely 1976; Yousfi et al., 2007).

Another popular shade tree species in the U.S. is river birch (*Betula nigra* L.). *B. nigra* is a short-lived tree (~ 40 years) with a unique form, texture, and reliable golden yellow fall color. Accounting for just over 25.1 million dollars in yearly U.S. horticultural sales in 2014, birch (*Betula* L.) trees represented 4.5% of deciduous tree sales. On the list of genera for the most sold deciduous shade trees, *Betula* ranks third, after maples (*Acer L.*) and *Quercus* (USDA Agriculture Census, 2014). Considering the vast number of species of maple, maple cultivars, and species of oak available, this figure representing the genus *Betula* is astounding. Unfortunately, like oaks, *B. nigra* is highly sensitive to elevated soil pH (Figure 1.2). The susceptibility of river birch to IFC in high soil pH limits its use in the landscape when soil pH is high, once again, creates a concern for sustainability.

Many of the current species of *Quercus* and cultivars of *B. nigra* used may not be the most sustainable options in landscapes with high pH soils, because they are not adaptable to a high soil pH and susceptible to IFC. (Denig et al., 2014; Hatch, 2015; Hawke, 1991; Neely 1996). For the species and cultivars that are adaptable to elevated soil pH don't exhibit IFC, the concern of overuse of these selections exists. The overuse of certain species or cultivars creates the absence plant genetic diversity, and a lack of plant genetic diversity leaves landscape unsustainable and vulnerable to attack by pests and pathogens (Santamour, 1990). Furthermore, the lack of plant diversity is poor for habitats of beneficial insects and wildlife. Thus, knowing more specifically the adaptability of species of oak and provenances of *B. nigra* to high soil pH,

additional plant-specific species recommendations could be made and promoted, ultimately leading to greater plant diversity and use in a landscape.

Limited studies investigating elevated pH on species of *Quercus* and *B. nigra* have been conducted, and much opportunity exists to determine and improve the selections the selections that are adaptable elevated soil pH and tolerant of IFC. Upper-limit pH studies on a broad range of species of *Quercus* and provenances of *B. nigra* should be conducted to determine the ranges of untapped genetic variation for these landscape taxon. Denig et al., (2014) provided maternal and parental adaptabilities for bur oak (*Quercus macrocarpa* Michx.), swamp white oak (*Quercus bicolor* Willd.), and chinquapin oak (*Quercus muhlenbergii* Engelm.) to alkaline soils; these species are commonly used in the landscape, because they are considered are adaptable to high soil pH compared to species of red oaks (*Quercus* sect. *Lobate*), specifically, pin oak (*Quecus palustris* L.). To understand pH adaptability for underused species of *Quercus* and *B. nigra* cultivars in the landscape, more research is needed. Again, a simple evaluation of the commonly cultivated species along with many uncommonly cultivated species at varying pH levels would be insightful. Provenances of the under-used species of *Quercus*, Texas red oak (*Quercus buckleyi* Nixon & Dorr) and Durand oak [*Quercus sinuata var. breviloba* (Torr.) C.H. Mull.] may provide tolerance to high soil pH in the landscape (Sternberg and Wilson, 1995). Furthermore, compared to cultivars of *B. nigra* originating from provenance south-eastern U.S. and Florida, tolerance to high soil pH may be found in provenances from uppermost northwestern distribution areas. More genetic diversity and pH adaptability in the landscape can be achieved if the identification of adapted species of *Quercus* and provenances of *B. nigra* to elevated soil pH is conducted, followed by information dissemination, and adoption by the landscape community occurs.

Histories, Native Distributions, and Potential of Two South-Central Texas Species of Oak in the Landscape

Texas red oak (*Quercus buckleyi* Nixon & Dorr)

Q. buckleyi was originally described in 1860 by Samuel Botsford Buckley but had accidentally applied the name *Quercus texana* Buckley (now Nuttall's oak) to his discovery (Griffin, 2008). In the last 125 years, many other taxonomic classifications have been applied to this species. Nixon and Dorr (1985) first proposed a new classification for Texas red oak as *Q. buckleyi*, because measurable differences between *Q. buckleyi* and *Q. texana* collections were observed. They indicated significant morphological differences in buds, leaves, twigs, cupules, and acorns between the two species. Furthermore, the native range of *Q. buckleyi* is further west in Texas than *Q. texana* (Stein et al., 2003). In addition to being confided with Nuttall's oak, some may confuse *Q. buckleyi* with Shumard's oak (*Quercus shumardii* Buckley) because the distributions overlap and hybrid swarms between the two species may exist in a central strip of the state (Simpson, 1988). On the other hand, Dorr and Nixon (1985) suggest since flowering phenology is vastly different between *Q. buckleyi* and *Q. shumardii*, they are reproductively isolated. Furthermore, Griffin (2008), has complied similarities and differences in plant size, bud, leaf, cupule, and acorn morphology between *Q. buckleyi*, *Q. shumardii*, and *Q. texana*, to serve as a better guide to when identifying these species.

Q. buckleyi is native to Texas (TX) and Oklahoma (OK), and some have indicated that its distribution extends into south-central counties in Kansas (KS), but this has never actually been confirmed (Kartez J.T. personal communication; USDA, 2018; Kenny and Wenzell, 2015). Confusion of the species distribution originated when Kartesz removed its proposed expansion into Kansas based on a lack of physical evidence while other databases, USDA, (2018) and

Kenney and Wenzell, (2015), basing their information from Kartesz and Meachum (1999), did not. Even though *Q. buckleyi,* has not been confirmed in KS, collections of the species were found in Osage County, OK in the early 1900's by George Walter Stevens whom had originally misidentified the collections as *Q. shumardii* (Goodman et al., 1978). Osage County, OK borders KS counties of Cowley and Chautauqua Counties, where other predominant species of *Quercus* such as blackjack oak (*Q. marilandica* Münchh.), *Q. macrocarpa, Q. muhlenbergii, Q. alba,* Northern red oak (*Q. rubra* L.), and *Q. shumardii* grow (Figures 1.3 and 1.4).

As a western upland species, Texas red oak does not grow to be as large as other oaks in *Lobate* (e.g. *Q. rubra, Q. shumardii,* and *Q. palustris*), but it may serve as a medium to large size landscape tree (10.5m) that provides long-lasting brilliant crimson red to burnt red fall color (Griffin, 2008; Sternberg and Wilson, 1995). In addition to being considered a very drought adaptable landscape species, some authors suggest *Q. buckleyi* is more tolerant of high pH soils compared to the other species in *Lobate* originating from eastern provenances, where the native soils are lower in pH (Balok and Hilaire, 2002; Griffin, 2008; Sternberg and Wilson, 1995). Its native hardiness distribution is from USDA Hardiness Zone 6b to 9a (Kartesz, 2015; Sternberg and Wilson, 1995). Kelaidis, (2011) claims that *Q. buckleyi* would be ideal for the surrounding Denver area from observations of a 15-year old, 6 m tall specimen that has been growing well in Denver, CO (zone 5b). Although *Q. buckleyi* may be considered marginally hardy in zone 5b, it has performed well in zone 6b. Several specimens and a nursery row can be found at the John Pair Horticulture Center (Haysville, KS), where the oldest is approximately 45 years old (Figures 1.5, 1.6, 1.7, and 1.8). One potential explanation for the lack of landscape use for this species, is the limited understanding of its hardiness colder than 6b, in addition to the fact few nurseries grow the species. Figure 1.9 shows *Q. buckleyi* growing in zone 8a to 8b at Joshua Springs Park

and Preserve, Kendall Co., TX and rich populations can be found in Lost Maples State Natural Area, Bandera Co., TX.

Durand oak [*Quercus sinuata* Walter var. *breviloba* (Torr.) C.H. Mull.]

Like *Q. buckleyi*, much confusion around typifying, Durand oak [*Q. sinuata* Walter var. *breviloba* (Torr.)] has surrounded this species for roughly 150 years. This species was first described in 1858 by an American botanist, John Torrey, as *Q. obtusifolia* var. *breviloba* (Sudworth, 1897). Over the next 125 years, many other taxonomic classifications were applied to this species as *Quercus durandii* and *Quercus. san-Sabena*, and later classified as variety of *Q. sinuata* (Dorr and Nixon; 1985; Ward 2007). Furthermore, *Q. sinuata* var. *breviloba* is often confused with another variety, bastard oak (*Quercus sinuata* var. *sinuata*) (Stein et al., 2003). The major difference between the two varieties is overall plant size; where *Q. sinuata* var. *breviloba* can be a clonal shrub or small tree (17 m), and *Q. sinuata* var. *sinuata* grows as a large (29 m) single or multi-stemmed tree (Stein et al., 2003). In addition to a difference in overall plant size, small morphological differences such as leaf size and shape are observable. *Q. sinuata* var. *breviloba* has more prominent, sinuated margins compared to *Q. sinuata* var. *sinuata*, however much morphological variation may exist making the difference between the two varieties difficult to discern (Simpson, 1998; Stein et at., 2003).

Furthermore, the distribution of these two varieties is distinct. *Q. sinuata* var. *breviloba* is found in the north and southcentral regions of TX, and southcentral OK preferring rich alluvial and limestone soils. An isolated population of *Q. sinuata* var. *breviloba* was found as far north as mid-west OK(Taylor, 2011). *Q. sinuata* var. *sinuata*, the eastern counterpart, spans several states from southeast TX to North Carolina (Stein et at. 2003). Rich populations of *Q. sinuata* var.

breviloba can be found in the Edwards Plateau, TX (Figure 1.12, 1.11, and 1.12), but can be confused with lacey oak *Q. laceyi* (Small), (Simpson, 1998; Nixon and Muller 1992).

Q. sinuata var. *breviloba* is even less common than *Q. buckleyi* as a landscape tree; some may not have even heard of the species. A couple reasons may attribute to its lack of use in the landscape, which include its gnarly form and potential to sucker which would make it difficult to grow uniformly in a nursery; or, because its fall color may not be as ornamentally desirable compared to other species of *Quercus* (Stein et al., 2003). Another potential reason for its lack of use is that it may not be cold hardy. The furthest northern hardiness zone where the species natively grows is 7a (Taylor, 2011). Even though not much is known about this species, Sternberg and Wilson (1995) suggest *Q. sinuata* var. *breviloba* is a species of interest because it may be pH adaptable. Without knowing much information regarding its ease of cultivation, fall color, cold hardiness, and adaptability to high soil pH, more attention should be given to this to determine its application and adaptability for landscape use.

Abundance, Oxidation Status, and Forms of Soil Fe

Fe is the fourth most abundant element on earth where the lithosphere alone contains 5.6% (Havlin et al., 2005). The issue limiting plant growth is not the abundance of Fe, rather the low availability to plants. In aerated conditions and a pH physiological suitable for plant growth, the concentrations of soluble Fe is no more than 10^{-15} M (Marschner, 2011). Fe is a transition element found as either Fe^{+3} (ferric) or Fe^{+2} (ferrous) in the soil. The oxidation state of Fe is highly dependent on abiotic and biotic factors such as soil moisture, aeration, soil type, soil chemistry, oxidation status of the soil, other minerals, and the soil microbial community (Marschner, 2011).

To fully understand the dynamics of Fe in the soil, an understanding of its various forms must been considered. Fe is found in primary materials; these are igneous and metamorphic rocks such as hornblende, biotite, and chlorite where it exists in the lowest oxidation state (Fe^{+2}) (Brown and Holmes, 1957). Secondary materials are created by biological weathering, where Fe is normally in the highest oxidation state (Fe^{+3}), but its oxidation state also depends on the type of parent material it is incorporated (Brown and Holmes, 1957).

The hydrolyzed inorganic forms of Fe include the Fe oxide: ferrihydrite, $Fe(OH)_{3\,(amorphous)}$ which constitute a large portion of the available Fe-pool in the soil (Lindsay, 1991). Fe solubility depends on the oxidation/reduction state of the soil and its different forms (Fageria et al., 1990). In oxidized soils if soluble $Fe(OH)_{3\,(soil)}$ is present, it quickly precipitates to $Fe(OH)_{3\,(amorphous)}$ in the presence of oxygen. In moderately oxidized soils, the dominant form is ferric oxyhydroxide [$Fe_3(OH)_8$], and in the reduced soils, $Fe(OH)_2^+$ or $Fe(OH)_3$ are relatively stable because of high moisture, lack of abiotic oxidizing factors, and low soil oxidation potential (<0.2 volts) (Fageria et al., 1990). Even though inorganic Fe complexes are present, the dominant forms of soluble Fe complexes in the soil are chelates of Fe, because the simple inorganic cationic forms (Fe^{+3} and Fe^{+2}) are readily hydrolyzed less soluble inorganic complexes ($Fe(OH)_2^+$, $Fe(OH)_3$, and $Fe(OH)_4^-$) (Fageria et al., 1990; Hinsinger,1998). Furthermore, the organic fraction of the soil alters the bulk of soluble Fe to a very large extent (Marschner, 2011). The organic fraction that alters soil Fe can be comprised of non-plant-derived organic acids or plant-derived metabolites. Some of these chelators are citric acid, which forms Fe-citrate when Fe phosphate is solubilized, and oxalic acid (Cesco et al., 2010; Lindsay, 1988). Other chelators may be flavonoids and phenols that can either be released actively or passively by the root

(which may be classified as phytosiderophores), during root decomposition, or by root injury (Cesco et al., 2010; Cesco et al., 2012; Maranjo-Arcos and Bauer, 2016: Shaw et al., 2006).

Factors Affecting Soil Fe Availability

Soil pH

The solubility of Fe^{+3} decreases by a factor of 1000 above a soil pH of 4 (Colombo et al., 2014; Lindsay, 1984). At a near neutral pH (7), Fe availability is generally very low, due to the high oxidation potential in the presence of oxygen. Fe^{+3} will hydrolyze and then precipitate from solution as Fe hydroxides or oxyhydroxides (Colombo et al., 2014). Furthermore, when the pH of an aerobic soil rises above 7, the estimated concentration or plant-available inorganic Fe is 10^{-10} M or 10,000 to 100,000 greater than what is needed for plant growth (Boukhalfa and Crumbliss, 2002). Römheld and Marschner (1986) estimated that for Fe not to be limiting plant growth, the concentration of inorganic Fe must be within the range of 10^{-5} to10^{-4} M. At elevated pH levels, the Fe forms that are generally found are compounds such as Fe_2O_3, carbonates, phosphates, and Fe^{+3} hydroxides [$Fe(OH)_2$], which are not taken up efficiently by plants (Ozores-Hampton, 2013).

Even though species of *Quercus* and *B. nigra* exhibit a wide scope of unique forms, textures, fall colors, and winter interests, many of these species may grow but perform poorly in the landscape when soil chemistry is unsupportive of plant growth and development. Neutral, mildly-alkaline, to alkaline soils have induced IFC in susceptible species in the landscape (Carlson, 2003). Unfortunately, urban soils generally may have elevated pH levels (range 6.8 to 9.8 with a mean on 8.7) from construction-related activities from the addition of bicarbonates to the soil (Jim, 1997). *B. nigra* and *Q. palustris* may display IFC in soils as low as pH 6.5 (Dirr, 2011; Neely, 1996). Depending on the species and soil pH, the degree of IFC expression is

caused by the lack in availability of soil Fe, where another micronutrient, manganese (Mn) can be limited, but is generally thought to play a minor role with IFC in species of *Quercus* (Neely, 1996). Furthermore, IFC expression depends on how well the species can cope with the lack of Fe and Mn availability (Brandt and Hartmann, 1998). While an Fe deficiency is often accredited to be the primary cause of IFC in species of *Quercus* and *B. nigra*, Mn deficiency may also be involved in the expression of the nutritional disorder (Carlson, 2003). Not only is IFC visually displeasing, it significantly affects the tree's health, specifically the ability to cope with biotic and abiotic stressors (Ward-Gauthier, 2013).

Lime

Bicarbonate (HCO_3^-) concentration along with high soil pH, exacerbates symptoms of IFC, because bicarbonates buffer high pH soils, diminishing Fe solubility and ability for root optimal root ferric chelate reductase (FCR) performance (Lucena, 2000; Lucena et al., 2007). Bicarbonates are strong bases and are produced when carbon dioxide (CO_2) is dissolved in water forming the weak acid, carbonic acid (H_2CO_3), which can then dissociate into HCO_3^- and H^+. When both HCO_3^- and H_2CO_3 are present in similar concentrations, strong buffering is achieved around a pH around 6.35 (Lucena, 2000). If a substrate is high in pH and is buffered against any rapid changes in chemistry, plant response to Fe deficiency may be negatively impacted, because it requires significantly greater proton extrusion from the roots to lower the rhizosphere pH.

Impact of Bicarbonates on Root and Xylem Tissues

Organic acids have shown to increase Fe mobilization in the soil and achieve apoplastic buffering when greater concentrations of soil-based HCO_3^- anions accumulate in the root (Chen et al., 2010; Kosegarten et al., 1998) The purpose of organic acid accumulation is to buffer the alkalization effect of HCO_3^-. The increase in concentration of HCO_3^- in the root may lead to a

decline in Fe uptake and translocation in the plant. Alhendawi et al., (1997) found that the pH of xylem sap of barley was most sensitive compared to sorghum and maize when supplied with $NaHCO_3$. Bialczyk and Lechowski (1995) found that xylem pH decreased and Fe concentration increased when HCO_3^- was added to a nutrient solution which is contrary to what Alhendawi et al., (1997) reported. The reason for the decrease of xylem sap pH was related to the influx of organic acids (citrate and malate) to buffer against the effect of HCO_3^- on xylem pH.

Fe Acquisition and Transport

Uptake

There are two major strategies plants use for Fe acquisition under Fe deficiency; Strategy I and II. Strategy I plants include dicotyledonous and non-graminaceous monocotyledonous species (De Vos et al., 1986). Plants using the Strategy II approach include graminaceous species, many of them having great agronomic influence in the world food trade market (Marschner, 2011).

When Strategy I species experience Fe deficiency, three main steps are induced to facilitate Fe uptake: a net efflux of hydrogen atoms (H^+) and phenolic excretion, an increase in fine-root FCR activity, and the expression of membrane bound Fe transporters (IRTs) (Marschner, 2011). The first step for soil Fe acquisition is the induction of H^+- ATPases to facilitate Fe uptake. ATPases actively pump H^+ atoms to acidify the rhizosphere; H^+ atoms displace adsorbed Fe^{+3} from soil particles into the rhizosphere (Marschner, 2011; Römheld et al., 1984). More simply, H^+ atom pumping creates a rhizosphere microenvironment where Fe^{+3} is free in the soil solution and is readily available to be chelated by various ligands (phenolic or flavonoid compounds) to improve the solubility of Fe^{+3} and a more efficient delivery to the reduction step of Fe^{+2} uptake (Chen et al., 2010; Maranjo-Arcos and Bauer, 2016; Marschner,

2011). This mechanism has been observed in cork oak (*Quercus suber* L.) grown in hydroponic culture. After 46 d of growing in a Fe-deficient culture, cork oak seedlings had successfully decreased solution pH from 6.0 to 3.5 where the control solution remained relatively unchanged, and when supplied with a fresh culture at 6.0 pH after five days, the solution pH dropped to 3.8 (Gogorcena et al., 2001). On the other hand, some plant species do not utilize the rhizosphere acidification mechanism. Under Fe-deficiency, farkleberry (*Vaccinium arboretum* Marshall), a Fe-inefficient species, and eight cultivars of Southern highbush blueberry (*V. ashi* x *corymbosum*), a Fe-efficient hybrids, do not utilize the rhizosphere acidification mechanism under Fe-stress. (Nunez et al., 2015). Furthermore, it is proposed that *Q. rubra* and silver birch (*Betula pendula* Roth.) use the rhizosphere acidification mechanism under Fe deficiency, and is a common mechanism for many tree species (Gogorcena et al., 2001; Ohno, 1989; Rosenvald et al., 2011; Venturas et al., 2014). The scientific community has not attempted to determine which or if popular species of *Quercus* and *B. nigra* cultivars utilize the Strategy I rhizosphere acidification mechanism.

Strategy I species may use the rhizosphere acidification mechanism to aid Fe^{+2} uptake, but the most crucial step in the process in Fe^{+2} uptake is Fe^{+3} reduction to Fe^{+2} via transmembrane proteins called FROs (Maranjo-Arcos and Bauer, 2016). These proteins can reduce either free Fe^{+3} or Fe^{+3}- ligand complexes (Marschner, 2011). When whole root systems of *Q. suber* were stained, it was revealed that Fe-deficient seedlings had a 4.5 times increase in FCR activity in the fine-root regions compared to the control (Gogorcena et al., 2001). Bond (1998) was unable to find any difference in FCR activity between seedlings of *Q. macrocarpa* and *Q. bicolor* and grown in nutrient cultures with varying Fe^{+3} concentrations. However, since

data were supported by limited replications, it is difficult to discern the qualitative differences in FCR activity between the two speces.

The last step in soil-Fe uptake involves the transport of Fe^{+2} into the epidermal cells (Marschner, 2011). Under Fe deficiency, arabidopsis (*Arabidopsis thaliana* L.) induces the expression of a transmembrane divalent cation transporter (IRT1), which transports Fe^{+2} into the plant (Dubeaux et al., 2015; Vert et al., 2002). Since IRT1 is a divalent cation transporter, Fe^{+3} and other trivalent cations are unable to be absorbed. Also, IRT1 can transport other divalent metals such as Mn, Zn, and Co, which if these exist in excess in the soil, they may increase competition for the absorption of Fe^{+2} (Dubeaux et al., 2015).

Unlike Strategy I, Strategy II species only require two steps for Fe^{+3} uptake: release of plant phytosiderophores and transport into the epidermal cells (Maranjo-Arcos and Bauer, 2016). The first step of Fe acquisition in graminaceous species. involves release of phtyosiderophores from transmembrane transporter proteins encoded by transporter of mugineic acid family phytosiderophores (*TOM*) genes in rice (*Oryza sativa* L.) and common barley (*Hordeum vulgare* L.); the over expression of these genes increases the net efflux of chelators and provides better tolerance to Fe deficiency (Nozoye et al., 2011). The chelators exuded in the highest concentrations from the root of Strategy I species are deoxymugineic acids which all increase the solubility of Fe^{+3} in the rhizosphere (Kim and Guerinot, 2007).

Like Strategy I, Strategy II species use a transporter to transport Fe into the epidermal cells. The major difference is that the root plasma membrane transporters in Strategy II species YSL1 (YELLOW STRIPE1) transports Fe^{+3} instead of Fe^{+2} in maize (Curie et al., 2001). By this, Strategy II species can completely mitigate the Fe reduction step required by Strategy I species. Marschner (1995) suggests that plants utilizing the Strategy II Fe deficiency response mechanism

are more efficient at acquiring Fe from the soil compared to Strategy I species, because Strategy II species can uptake Fe^{+3}-ligands skipping Fe^{+3} reduction. Furthermore, *Oryza sativa* is adapted to take up either oxidation state on Fe, because it normally grows in waterlogged soils where Fe^{+2} is naturally abundant (Ishimaru et al., 2006).

Transport

Once $Fe^{+2/+3}$ is transported into the epidermal cells by IRT proteins in Strategy II species and proteins similar to YSL in Strategy I species, it complexes with citric acid (CA) or nicotinamide (NA) chelators, and the complex proceeds to move from the cortex, to the endodermis, and finally into stele via the symplastic pathway by diffusion (Kim and Guerinot, 2007). When the complex reaches the xylem by apoplastic movement from the stele, it is loaded by FERROPORTIN 1 (FPN1) in *A. thaliana* (Maranjo-Arcos and Bauer, 2016; Mckie et al., 2000). If Fe is not absorbed into the epidermis, it moves via the apoplastic pathway until it is blocked by the Casparian strip (Morrissey et al., 2009). Here Fe may be oxidized by various compounds, precipitated into the root apoplastic tissue, and then re-utilized after it is re-solubilized by phenolic compounds and organic acids (Guerinot, 2010).

Under optimal conditions (pH of 5.5 to 6) in the xylem sap, CA complexes any unbound Fe, and the complex follows the transpiration stream of the shoot, completing its journey into the leaf cytoplasm (Rogers and Guerinot, 2002). If Fe is no longer in demand for developing plant tissues or reproductive organs, it will precipitate into shoot or leaf apoplastic space until it is needed (Kim and Guerinot, 2007).

When needed in sink tissues, Fe can be remobilized and moved via the phloem loading and unloading pathway (Kim and Guerinot, 2007). Xylem is a poor pathway for Fe to reach sink tissues and developing parts of the plant, because it is undeveloped unlike phloem tissue in these

regions (Lanquar et al., 2005). Fe is usually chelated in the phloem sap as it is normally neutral or slightly-alkaline. IRON TRANSPORT PROTEIN (IRT) or NA chelate Fe^{+3} binds to $Fe^{+2/+3}$, respectively, to facilitate movement of Fe through the phloem (Kruger et al., 2002; von Wiren et al., 1999). Furthermore, the upregulation of NICOTINAMIDE SYNTHASE 3 (NAS3) in the phloem has shown to increase Fe concentrations in reproductive organs (Klatte et al., 2009). The main sink tissues for Fe are the chloroplasts and mitochondria, because the abundance of Fe in these organelles dictates photosynthesis, the electron transport chain (ETC), and enzyme synthesis (Maranjo-Arcos and Bauer, 2016). FERRIC CHELATE REDUCTASE 7 (FRO7) reduces Fe^{+3} before PERMEASE IN CHLROPLAST 1 (PIC1) transports Fe to the chloroplast (Jeong et al., 2008; Duy et al., 2007; Duy et al., 2011). For long- term storage, Maranjo-Arcos and Bauer (2016) summarize the import and export into cell vacuoles via the control of Fe transporter genes, the incorporation of Fe into natural resistance macrophage proteins, and chelation by phytates.

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Figure 1.1 Foliar chlorosis on Northern red oak (*Quercus rubra* L.) growing on Purdue University's campus, West Lafayette, IN taken 29 June 2017.

Figure 1.2 Extreme dieback and symptoms of foliar chlorosis on river birch (*Betula nigra* L.) growing in an urban area in Kansas City, MO taken 22 May 2016.

Figure 1.3 Native stand of oaks (*Quercus* L.) in Osage County Oklahoma taken 11 Nov. 2017. A stately red Shumard's oak (*Quercus shumardii* Buckley) is the in the center of the photograph.

Figure 1.4 Fall foliage color on a Shumard's oak (*Quercus shumardii* Buckley) in Osage County OK taken 11 Nov. 2017.

Figure 1.5 Nursery row of Texas red oaks (*Quercus buckleyi* Nixon & Dorr) from multiple seed TX sources at the John C. Pair Horticulture Center, Haysville, KS taken 11 Nov. 2017. Note the variation in fall color; some later coloring trees will hold fall color until early December.

Figure 1.6 Fall foliage color of two Texas red oaks (*Quercus buckleyi* Nixon & Dorr) at the John C. Pair Horticulture Center, Haysville, KS planted circa 1974. Notice differences in fall color timing; tree on the far left colored much sooner than the tree on the right. Photo courtesy of the late Dr. John C. Pair. (Pair, n.d.).

Figure 1.7 Specimen Texas red oak (*Quercus buckleyi* Nixon & Dorr, approx. 15 yr old) at the John C. Pair Horticulture Center, Haysville, KS after transplantation circa 1991. Photo courtesy of the late Dr. John C. Pair. (Pair, 1991).

Figure 1.8 Specimen Texas red oak (*Quercus buckleyi* Nixon & Dorr, approx. 45 yr old; same tree from Figure 1.7) at the John C. Pair Horticulture Center, Haysville, KS. Photo courtesy of Dr. Jason J. Griffin (Griffin, 2014). This specimen consistently holds crimson-red foliage until early Dec. Notice fall color of sawtooth oak (*Quercus acutissima* Carruth.) just in the background

Figure 1.9 Texas red oak (*Quercus buckleyi* Nixon & Dorr) at Joshua Springs Park and Preserve, Kendall Co., TX taken 15, Oct. 2015, from which acorns where collected.

Figure 1.10 Durand oak [*Quercus sinuata* var. *breviloba* (Torr.) C.H. Mull.] at Lost Maples State Natural Area, Bandera Co., TX taken 15, Oct. 2015, from which acorns were collected in 2015 and 2016.

Figure 1.11 Form of Durand oak [*Quercus sinuata* var. *breviloba* (Torr.) C.H. Mull.] at Lost Maples State Natural Area, Bandera Co., TX taken 15, Oct. 2015, from which acorns were collected in 2015 and 2016.

Figure 1.12 Bark color and texture of Durand oak [*Quercus sinuata* var. *breviloba* (Torr.) C.H. Mull.] at Lost Maples State Natural Area, Bandera Co., TX taken 15, Oct. 2015, from which acorns were collected in 2015 and 2016.

Chapter 2 - Assessing the Tolerance of Texas Red Oak, Durand Oak, and Pin Oak to Foliar Chlorosis in High pH Substrate

Abstract

Pin oak (*Quercus palustris* L.) is known to develop iron (Fe) deficiency-induced foliar chlorosis (IFC) when grown in alkaline soils. In contrast, species and provenances endemic to alkaline soils do not always display this chlorosis. More environmentally tolerant and aesthetically pleasing taxa could be used if they are first screened to determine their adaptability to high pH soils. Three experiments were conducted to evaluate wild collected Texas red oak (*Q. buckleyi* Nixon & Dorr) and durand oak [*Q. sinuata* Walter *var. breviloba* (Torr.) C.H. Mull.] with landscape collections of *Q. palustris* to determine the extent of foliar chlorosis when grown in an elevated pH substrate. In Expt. 1 acorns of each species were planted in 3.8 L containers in a pine bark substrate amended with either standard (2.4 $kg \cdot m^{-3}$) or high (11.9 $kg \cdot m^{-3}$) rate of dolomitic lime to create control and elevated pH substrate treatments, respectively. Twice-weekly 120 mL flowable 4.77% $CaCO_3$ drenches were used to maintain pH in elevated substrate treatments, and periodical aluminum sulfate drenches were applied to reduce pH of control substrate treatments in 2017. In Expt. 2, acorns of each species were planted in 3.8 L containers in a pine bark substrate and received twice-weekly 120mL drenches of either 0.00, 0.200, 1.97, and 4.77% flowable $CaCO_3$ drenches. Plants were overwintered and potted into 11 L containers and continued to receive drenches of flowable $CaCO_3$ or aluminum sulfate to raise or reduce substrate pH as needed. Two seed sources of *Q. palustris* developed IFC and we observed a range of reduced mean leaf SPAD readings (14.0 to 18.9) and low total chlorophyll concentrations (6.36 to 11.9 $\mu g \cdot cm^{-2}$) when grown in the elevated compared to non-chlorotic seedlings SPAD (36.7 to 38.7) and total chlorophyll (24.2 to 36.1 $\mu g \cdot cm^{-2}$) in the control pH

substrate. There were no symptoms of IFC or differences in SPAD in *Q. buckleyi* and *Q. sinuata* var. *breviloba* when grown in either the elevated pH substrate. We observed range of leaf SPAD readings for non- chlorotic *Q. buckleyi* and *Q. sinuata* var. *breviloba* (31.7 – 36.0 and 32.5 – 40.1, respectively). Similarly, total chlorophyll of *Q. buckleyi* and *Q. sinuata* var. *breviloba* was not affected by the elevated substrate pH where too we observed a range (24.3 to 36.0 $\mu g \cdot cm^{-2}$ and 23.1 to 36.6 $\mu g \cdot cm^{-2}$, respectively). We observed overlapping ranges for mean total leaf Fe content for between chlorotic and non- chlorotic leaves of *Q. palustris* seedlings (12.8 to 45.5 $mg \cdot kg^{-1}$, chlorotic and 35.3 to 64.9 $mg \cdot kg^{-1}$, non- chlorotic) and a large variation of non-chlorotic leaves *Q. buckleyi* and *Q. sinuata* var. *breviloba* seedlings (41.7 to 208 $mg \cdot kg^{-1}$ and 27.4 to 134 $mg \cdot kg^{-1}$, respectively). Data herein suggests that *Q. buckleyi* and *Q. sinuata* var. *breviloba* may be less susceptible Fe-induced interveinal foliar chlorosis by sequestering greater amounts of substrate Fe and having increased Fe use efficiency when compared to *Q. palustris* growing in a high pH substrate. Field evaluations with considerations of provenance performance in different hardiness zones should be used to determine their potential as more suitable species for use in landscapes with high pH soils.

Introduction

Oaks (*Quercus* L.) are an integral part of American history and serve as valued, dominant, and picturesque shade tree specimens in landscapes. With over 1,600 nurseries growing oak trees and 3.3 million plants sold, oak sales in the United States (U.S.) topped $105 million and accounted for 18.8% of all deciduous shade tree sales in 2014 (USDA Agriculture Census, 2014). One group, the red oaks of North America (*Quercus* sect. *Lobate*), are ornamentally desirable because they offer crimson, red, burnt orange, and russet fall colors in U.S. Department of Agriculture (USDA) 2012 Hardiness Zones 6 to 3 landscapes (Sternberg and

Wilson, 1995). Unfortunately, many species in the *Lobate* section develop Fe deficiency induced foliar chlorosis (IFC) when planted in soils with high pH, because as soil pH increases, the availability of iron for root uptake diminishes (Lindsay and Schawb, 1982). From a series of soil tests of 100 urban sites, Jim (1997) revealed that the addition of carbonates (CO_3^{-2}) from construction related activities can increase soil pH to alkaline levels (mean 8.7). Furthermore, high calcium carbonate ($CaCO_3$) content in soils can intensify symptoms of IFC in plants, because high soil bicarbonates (HCO_3^-) diminish the Strategy I response to Fe deficiency (Mengal et al. 1984). When soil HCO_3^- concentration is high, the soil is buffered from any potential change in pH thus diminishing the effect of Strategy I rhizosphere acidification mechanism used by many woody plants, including cork oak (*Quercus suber* L.) and presumably red oak (*Quercus rubra* L.), under Fe deficiency (Brancadoro et al., 1995; Gogorcena et al., 2001; Ohno, 1989; Romera et al., 2008; Rosenvald et al., 2011; Venturas et al., 2014). Additionally, Fe reduction at the root rhizosphere interface is another crucial step before Fe uptake, and soil bicarbonate can impede optimal root ferric chelate reductase (FCR) performance (Lucena, 2000; Lucena et al., 2007).

The most frequent symptoms of IFC in *Quercus* occur with *Quercus palustris* which is the most popular species for Midwestern landscapes, because it transplants easily, grows rapidly and large, and has reliable fall color (Dawson and Hauer, 1996; Harrell et al., 1984; Harris and Day, 2010; Neely, 1976; Sternberg and Wilson, 1995; Van Sambeek et al., 2017). *Q. palustris* is native to the bottomlands of northeastern and northcentral U.S. and thrives in acidic soils, but grows poorly in soils with high pH and $CaCO_3$ content (Kabrick et al., 2005). Although selection for provenances of *Q. palustris* tolerant to IFC has been conducted, no practical follow through with the provenances showing promise arose from the findings, and susceptible *Q. palustris* are

still being planted (Berrang and Stiner, 1980; Kriebel, 1993). In recent years, the development of hybrid cultivars of *Quercus* with tolerance to IFC has been slow because, crosses between two tolerant species does not necessarily result in progeny with tolerance, viable methods of asexual propagation techniques are still rudimentary and not cost effective, and sexual propagation remains the standard nursery practice (Chalupa, 1988; Chalupa, 1993; Denig et al., 2013; Denig et al., 2014).

In previous work, Bond (1998) explored Fe efficiency among provenances of bur oak (*Q. macrocarpa* Michx.) and swamp white oak (*Q. bicolor* Willd.) in static solution culture. He concluded that *Q. macrocarpa* is was more Fe efficient than *Q. bicolor*. He also reported that provenances occurring in drier regions were more efficient than those from mesic climates. This is similar to the findings of Berrang and Stiner (1980) when they screened provenances of *Q. palustris* for pH adaptability. Denig et al., (2014) focused on developing pH adaptability through interspecific hybrids within *Quercus* sect. *Quercus* (white oaks). They found that the most adaptable progeny where those derived from the maternal parents of *Q. macrocarpa*, chinkapin oak (*Q. muehlenbergii* Engelm.), and ooti oak [English oak (*Q. robur* L.) × *Q. macrocarpa* × *Q. muehlenbergii*] when grown in peat-based substrate.

Q. macrocarpa and *Q. muhlenbergii* are acceptable alternatives for planting in landscapes plagued by high soil pH and $CaCO_3$, but these species in sect. *Quercus* do not develop desirable fall color compared to some species in sect. *Lobate* (Denig et al., 2014; Stein et al., 2003). A reasonable alternative to planting species of *Quercus* susceptible to IFC or species lacking striking fall color is diversifying the landscape with species that produce desirable fall color and possess tolerance to IFC. Shumard's oak (*Quercus shumardii* Buckley) of sect. *Lobate* is a common alternative with desirable red fall color that may possess better adaptability to high pH,

but has not been fully explored (Dirr, 2011; Kennedy and Krinard, 1985). To facilitate the selection of species of *Quercus* with greater tolerance to IFC it is assumed that interspecific variation for soil pH adaptability exists, because the distribution of species of *Quercus* encompasses many diverse edaphic habits within the U.S. (Denig et al., 2014; Kartesz, 2015; Stein et al., 2003). If species of *Quercus* native to limestone soils in warm and dry regions are considered to be screed for tolerance, we may be one step closer to revealing a species with better soil pH adaptability (Slessarev et al., 2016).

Texas red oak (*Q. buckleyi* Nixon & Dorr) is an underutilized southcentral upland species of sect. *Lobate* native to Oklahoma and Texas that produces long-lasting crimson red to burnt red fall color and may be more adapted to high pH soils because it naturally occurs in limestone soils (Griffin, 2008; Kartesz J.T. personal communication, Sternberg and Wilson, 1995). Although *Q. buckleyi* may be considered marginally hardy outside its native range, several specimens have performed well in USDA 2012 Hardiness Zones 6b at the John Pair Horticulture Center, Kansas State University (Haysville, KS) for approximately 45 years. Furthermore, a relatively unknown species in sect. *Quercus*, that is confined to the same distribution and soils as *Q. buckleyi*, is durand oak [*Quercus sinuata* Walter var. *breviloba* (Torr.) C.H. Mull.]. It may prove worthwhile to more closely examine *Q. sinuata* var. *breviloba*, because a lack of information regarding its cultivation, soil pH adaptability, and fall color exists.

The objective of this work was to investigate the development of IFC in containerized *Q. buckleyi, Q. sinuata* var. *breviloba,* and *Q. palustris* growing in an elevated pH substrates. Foliar nutrient and chlorophyll content along with plant growth characteristics were measured to determine each species relative tolerance to resisting IFC.

Materials and Methods

Two experiments were conducted during the course of this work. Experiment 1 was conducted during Summer 2016 and repeated in Summer 2017. Experiment 2 was conducted during the summers of 2016 and 2017. Both experiments were conducted in a polycarbonate greenhouse at the Kansas State University John C. Pair Horticultural Center (Haysville, KS).

Seed Source Collection

Expt. 1 (2016 and 2017)

Acorns of two sources of *Q. palustris* were collected in Fall 2015 for the Summer 2016 experiments. The first source was a bulk collection from two masting and visually healthy residential landscape trees in Wichita, KS (ICT) with no prior history of foliar chlorosis. Acorns of the second source came from a mature tree on the Kansas State University (KSU) campus that had received an injection of 20.37 kg solution of 0.0072% Fe (by wt.) (ferric sulfate tetrahydrate, Medi-Ject Tree Injection Systems, Lincoln, NE) by a local arborist company (Tree BioLogics Inc., Manhattan, KS) in the Spring 2015 due to a history of foliar chlorosis. Acorns of *Q. buckleyi* were also collected in Fall 2015 at Joshua Springs Park and Preserve (JSPP), Kendall Co., TX on 19 Oct. 2015 (Latitude: 29.884652, Longitude: -98.814332) from a single tree in a native stand. Acorns of *Q. sinuata* var. *breviloba* were obtained from Lost Maples State Natural Area (SNA) Bandera Co., TX on 19 Oct. 2015 and September 18, 2016 (Latitude: 29.815871, Longitude: -99.576307); also from a single tree in a native stand.

Acorns of the same species were collected again in Fall 2016 for the experiment conducted in Summer 2017. For this experiment, only one source of *Q. palustris* was collected, and it was from another masting specimen at KSU with a known history of foliar chlorosis. This was a different tree from the previous year collection, but it had also received a similar Fe sulfate

injection in Spring 2016. Acorns of *Q. buckleyi*, were collected from a single tree in a native stand at Kerr Wildlife Management Area (Kerr WMA), Kerr Co., TX on 18 Sept. 2016 (Latitude : 30.061576, Longitude : -99.518315). Acorns of *Q. sinuata* var. *breviloba* were collected from the same source as previously mentioned in Fall 2015.

The day after acorn collection, cupules were removed, acorns were rinsed with tap water, and float tested (Bonner and Vozzo 1987). Seeds were placed in 3.8 L polyethylene bags with eight 1.3 cm perforations to ensure adequate gas exchange. Each bag contained four sheets of moist paper towel, which were periodically re-wetted to maintain high humidity. Seed were stored in the dark at 3°C until planting.

Expt. 2 (2016 to 2017)

Acorns were collected from the same sources during the Fall 2015 collection described for Expt. 1: two sources of *Q. palustris* (ICT and KSU), one source of *Q. buckleyi* (Kendall Co., TX), and one source of *Q. sinuata* var. *breviloba* (SNA). Acorns were processed and stored as described for Expt. 1.

Study Initiation, Treatments, Experimental Design, and Data Collection

Expt. 1 (2016 and 2017)

Expt. 1. On 8 Apr. 2016 three acorns of a species were planted into each 3.8 L container (Classic 400, Nursery Supplies Inc.™, Chambersburg, PA) filled with a 3:1 (by vol.) pine bark (Hapi Gro® Composted Pine Bark, Hope Agri Products, Inc., Hope, AR) : perlite (Therm-O-Rock® Perlite, Therm-O-Rock. Inc., Chandler, AZ) substrate amended with 2.8 $kg \cdot m^{-3}$ Osmocote Classic® (14N-4.2P-11.6K) (Everris NA, Inc., Dublin, OH), and 0.5 $kg \cdot m^{-3}$ Micromax® (Everris NA, Inc., Dublin, OH). Substrate pH treatments were created by incorporating dolomitic lime (Deco® Lawnlime®, The Georgia Marble Co., Kennesaw, GA) at

2.4 $kg \cdot m^{-3}$ (control) or 11.9 $kg \cdot m^{-3}$ (elevated pH) rendering an initial substrate pour-through pH of 5.66 and 6.90, respectively. Five weeks after planting, all containers were thinned leaving the most vigorous seedling.

The following year, 3 Mar. 2017, three acorns of a species were planted into each 3.8 L container filled with a 9:1:4 (by vol.) bark (Yardcare™ Small Western Bark, Mountain West Products, Rexburg, ID.) : soil conditioner (Yardcare™ Soil PEP, Mountain West Products, Rexburg, ID.) : perlite. Macro- and micro-nutrient additions and dolomitic lime amendments were the same as in 2016. Initial substrate pour-through pH of the control and elevated pH treatments were 7.85 and 7.99, respectively. Five weeks after planting, all containers were thinned leaving the most vigorous seedling.

In both years, plants were grown on benches in a polycarbonate greenhouse under 50% shade cloth and exposed to natural photoperiod with temperatures set at 24 °C day/20 °C night. Plants were irrigated twice weekly with 120 mL tap water (Table 2.1) for nine weeks to allow the root systems to fill the containers. After nine weeks, plants were irrigated twice weekly with 120 mL solution of 0% or 4.77% liquid calcium (CalOx® pH, BioSafe Systems LLC., Hartford, CT) and 0.122% magnesium sulfate heptahydrate [($MgSO_4 \cdot 7H_2O$), PDC Brands™ Stamford, CT] in tap water to create a low and high substrate solution pH, respectively. Substrate solution pH and electrical conductivity (EC) were monitored weekly (2016) or biweekly (2017) using the pour-through technique (Wright, 1986). A change in pine bark supplier resulted in substrate pH much greater than desired in 2017. To remedy the high substrate solution pH, plants receiving 0% liquid calcium drench also received an acidifying drench of 60 mL tap water with 10.8% aluminum sulfate [$Al_2(SO_4)_3$; Voluntary Purchasing Groups Inc, Bonham, TX] every four weeks

to lower substrate pH. Fourteen weeks after planting all containers were top-dressed with 11 g of a controlled release fertilizer (Osmocote Pro® 19N-2.2P-6.6K).

Using a SPAD meter (SPAD-502, Konica Minolta, Inc.), SPAD values were taken bi-weekly beginning nine weeks after experiment initiation. Measurements were collected by recording one data point on the bottom right lobe on each of three most recently fully expanded leaves per plant. Leaf chlorophyll content was obtained by punching 16 mm^2 leaf discs from four of the most fully expanded leaves per plant the day before experiment termination. The leaf discs were taken from the bottom right lobe on each leaf. Eight leaf discs were placed in a 56 mL glass test tube with 16 mL *N*,*N*-Dimethylformamide (DMF; Fisher Scientific, Hampton, NH). Chlorophyll was extracted under dark conditions for 24 h. For the final 12 h of incubation, samples were placed on a platform shaker (Innova 2100, New Brunswick Scientific, Edison, NJ) at 80 rpm and 24 °C. Before spectrophotometric analysis all samples were homogenized at 860 rpm for 2 s (Vortex Genie 2, Fisher Scientific, Hampton, NH). Chlorophyll a and b were determined with spectrophotometric analysis using a Hitachi U1100 spectrophotometer (Hitachi, Ltd., Tokyo, Japan) at 647 and 664 nm, and equations derived by Porra et al., (1989).

After 20 (2016) and 25 (2017) weeks, the experiments were terminated and remaining data were collected. Data included height, stem caliper at substrate interface, leaf number, leaf size, and dry weight of stem and leaf tissue. Leaf size was determined using a leaf area meter (Model Li-3100C, Li-Cor®, Lincoln, NE). Dry weights were determined by placing tissue in a forced-air drying oven (Grieve SC-350 Electric Shelf Oven, Round Lake, IL) at 71°C for 6 d. Total C and N in leaf tissue was obtained with a C/N combustion analyzer (LECO TruSpec CN, LECO Corporation, St. Joseph, MI) by the Kansas State Soil Testing Laboratory (KSU, Manhattan, KS). Furthermore, phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg),

zinc (Zn), Fe, copper (Cu), manganese (Mn), and sulfate (SO_4) in leaf tissue was obtained from Perchloric digest [Inductively Coupled Plasma (ICP) Spectrometer, Model 720-ES ICP Optical Emission Spectrometer, Varian Australia Pty Ltd., Mulgrave, Vic. Australia] (Gieseking et al., 1935).

The experimental design was a randomized complete block design (RCBD) with a factorial arrangement of treatments (species x substrate pH). There were four (2016) or three (2017) seed sources comprised of three species in combination with two substrate pH levels creating eight (2016) or six (2017) treatments. Each treatment combination was initially replicated eleven (2016) or ten (2017) times, but some treatment replications were lost due to poor germination in both years. Therefore, in 2016, there were ten replications of (*Q. buckleyi* x elevated pH), and nine for (*Q. sinuata var. breviloba* x elevated pH), and in 2017 there were seven replications of (*Q. sinuata var. breviloba* x control pH), and eight for (*Q. sinuata var. breviloba* x elevated pH) used for statistical analysis. To generate sufficient leaf tissue for nutrient analysis, leaf discs of similar treatments were combined across replications resulting in five replications for this analysis. However, due to poor germination, there were ultimately four replications for *Q. sinuata var. breviloba* x elevated pH in 2016, and three replications of *Q. sinuata var. breviloba* x control pH, and five for *Q. sinuata var. breviloba* x elevated pH in 2017 used for statistical analysis. We analyzed data in SAS® University Edition (SAS Institute Inc., Cary, NC).When analyzing the effect of substrate pH treatment on a collection, data normality for data was tested using proc univariate, and if data were normal, they were subjected to an proc two sample ttest $P \leq 0.05$; if data were not normal, they were subjected to WILCoxon-Mann-Whitney test $P \leq 0.05$. When collections were compared within a substrate pH treatment, a one-way analysis of variance was conducted using general linear model (proc GLM). When

appropriate, means were separated at $P \leq 0.05$ using Fisher's Protected LSD. For leaf chlorophyll concentrations, a two-way analysis of variance using general linear model (GLM) was employed and means were separated at $P \leq 0.05$ using Fisher's Protected LSD.

Expt. 2 (2016 to 2017)

Expt. 2. On 8 Apr. 2016 three acorns of a species were planted into each 3.8 L container filled with a 3:1 (by vol.) pine bark (Hapi Gro® Composted Pine Bark) : perlite substrate amended with 2.4 $kg \cdot m^{-3}$ dolomitic limestone and the same macro- and micro-nutrient package described in Expt. 1. Initial pour-through substrate solution pH was 5.63. Five weeks after planting, all containers were thinned to one plant, leaving the most vigorous seedling.

Containers were placed on a gravel floor in the same greenhouse and under the same environmental controls as Expt. 1. Plants were irrigated twice weekly with 120 mL of tap water for nine weeks to ensure root systems filled the containers. After nine weeks, plants were watered twice weekly with a 120 mL solution of four rates: 0% (control), 0.200% (low), 1.97% (medium) or 4.77% (elevated) liquid $CaCO_3$ and 0%, 0.005%, 0.050%, and 0.122% $MgSO_4 \cdot 7H_2O$, respectively, in tap water. Substrate solution pH and EC were monitored every week using the pour-through technique. Fourteen weeks after planting, containers were top-dressed with 11 g of the same controlled release fertilizer in Expt. 1.

On 2 Sept. 2016, liquid calcium treatments were terminated and plants were moved outside to a gravel container production pad to harden off. Plants were overwintered in an unheated white polyethylene-covered hoop house. On 3 March 2017, all plants with existing substrate intact were potted into 11 L containers using the same substrate, lime, and nutrient packages as Expt. 1 (2017) and returned to the greenhouse. Plants were irrigated twice weekly with 360 mL tap water and given another nine weeks to establish in their new containers before

liquid $CaCO_3$ drenches resumed as described above. Substrate solution pH and EC were monitored every two weeks, and SPAD was taken three times in 2017 using the same methods as described in Expt. 1. Furthermore, similar to Expt. 1 (2017), the substrate solution pH was greater than desired. To lower pH, the plants receiving 0% liquid $CaCO_3$ received 180 mL drenches 10.8% $Al_2(SO_4)_3$ every four weeks. Fourteen weeks after planting in 2016 and 2017, all containers were top-dressed with 11 g and 33 g, respectively, of a controlled release fertilizer (Osmocote Pro® 19N-2.2P-6.6K).

Twenty-four weeks after planting, data were collected and the experiment was terminated. Data included final SPAD values, final pH and EC, and leaf chlorophyll a and b concentration; all parameters were taken using the same methodology described in Expt. 1. Growth data included height, stem caliper at soil interface, leaf number, leaf size, and dry weight of stem and leaf tissue. Dry weights and tissue nutrient analysis were collected following the same procedure in Expt. 1.

The experimental design was a RCBD with a factorial arrangement of treatments (species x substrate pH). There were four collections in combination with pH levels creating sixteen treatments. Each treatment combination was initially replicated eight times, but some treatment replications were lost due to poor germination in 2016. Therefore, there were seven replications of (*Q. palustris* ICT x control pH), five for (*Q. sinuata* var. *breviloba* x control pH), and five for (*Q. sinuata* var. *breviloba* x low pH) used for statistical analysis. To generate sufficient leaf tissue for nutrient analysis, leaf discs of similar treatments were combines across replications resulting in originally four replications for this analysis. Since some treatment replications had been lost or not combinable due to the odd number of replications there were three combined replications of (*Q. palustris* ICT x control pH), two for (*Q. sinuata* var. *breviloba* x control pH),

and two for (*Q. sinuata* var. *breviloba* x low pH) used for statistical analysis. The effect of substrate pH analyzed within a collection using a one-way analysis of variance with using general linear model (proc GLM). When appropriate, means were separated at $P \leq 0.05$ using Fisher's Protected LSD. For leaf chlorophyll concentrations, a two-way analysis of variance using general linear model (GLM) was employed and means were separated at $P \leq 0.05$ using Fisher's Protected LSD.

Pest Control

One week after planting of all experiments, containers received a drench of (R,S)-2-[(2,6-dimethylphenyl)-methoxyacetylamino]-propionic acid methyl ester (mefenoxam) (0.13 $mL \cdot L^{-1}$ reverse osmosis (RO) water, Syngenta® Greensboro, NC) as a preventative measure to combat root diseases. Weekly rotations of foliar applications of spinosyn A and D (spinosid) (0.8 $mL \cdot L^{-1}$ RO water, Dow AgroSciences™, Indianapolis, IN), azadirachtin (0.62 $mL \cdot L^{-1}$ RO water, Certis USA, Columbia, MD), 2-[1-Methyl-2-(4-phenoxyphenoxy)ethoxy]pyridine (pyriproxyfen) (0.018 $mL \cdot L^{-1}$ RO water, Valent USA®,Walnut Creek, CA), and potassium salts of fatty acids (13.2 $mL \cdot L^{-1}$ RO water, Dow AgroSciences™, Indianapolis, IN) was utilized to prevent typical greenhouse pests such as western flower thrips [*Frankliniella occidentalis* (Pergande)], fungus gnats (*Bradysia* spp.), whiteflies [*Trialeurodes vaporariorum* (Westwood)], cabbage looper [*Trichoplusia ni* (Hübner)], and armyworm moth [*Pseudaletia unipuncta* (Haworth)]. However, some *F. occidentalis* feeding on meristem tissue in 2016 and foliar feeding of lepidopterous larvae in 2016 and 2017 was observed. Also, tetrachloroisophthalonitrile (chlorothalonil) (1.69 $g \cdot L^{-1}$ RO water, Syngenta® Greensboro, NC) and methyl 2-(1-(4-chlorophenyl)pyrazol-3-yloxymethyl)-N-methoxycarbanilate (pyraclostrobin) (0.21 $g \cdot L^{-1}$ RO water, BASF, Florham Park, NJ) were rotated every four weeks to prevent fungal development on developing foliage.

Results

Expt. 1 2016

Over 132 d, substrate leachate pH for the control treatment (mean pH = 5.58) declined over the course of the experiment (Figure 2.1A). Substrate leachate pH values for the elevated treatment (mean pH = 6.72) declined after 76 d, but gradually increased with twice-weekly drench applications of 4.77% flowable $CaCO_3$. There was a significant difference in final leachate pH between the treatments (control = 5.30, elevated = 6.87, $P = 0.0067$). Leachate electrical conductivity (EC) values for control and elevated pH substrates increased approximately six-fold over the course of the experiment, and upon termination, leachate EC values between substrate pH treatments were not statistically different (control = 5.09, elevated = 5.80 $mS \cdot cm^{-1}$, $P = 0.9155$) (Figure 2.1B).

The substrate pH treatment influenced all growth parameters (height, stem diameter, leaf number, leaf area, and leaf and shoot dry weight), SPAD readings, and leaf chlorophyll a and b concentrations for each collection were affected differently (Table 2.2). Similarly, all macro- and micro-nutrients were affected by the substrate pH treatment (Table 2.2). Reductions in plant height by 14% and 43% were observed for *Q. palustris* (ICT and KSU) respectively, in the elevated pH substrate (Table 2.3). Neither *Q. buckleyi* nor *Q. sinuata* var. *breviloba* growing in the elevated pH substrate were reduced in height (Table 2.3). An identical trend was observed for stem diameter. *Q. palustris* ICT and KSU seedlings growing in the elevated pH substrate had 20% and 45% reductions, respectively (Table 2.3), whereas *Q. buckleyi* and *Q. sinuata* var. *breviloba* were unaffected. Both, *Q. buckleyi* and *Q. palustris* (KSU) experienced dramatic reductions in leaf number when grown in the elevated substrate pH treatment (Table 2.3). However, the *Q. palustris* (ICT) and *Q. sinuata* var. *breviloba* leaf numbers were unaffected. *Q.*

palustris (ICT and KSU) in the control pH substrate had the greatest total leaf area compared to the two Texas natives, but when grown in the elevated pH substrate, total leaf area was reduced by 42% and 78%, respectively. No significant reductions in leaf area were observed for either *Q. buckleyi* or *Q. sinuata* var. *breviloba* in the elevated pH substrate. Total leaf dry weight and shoot dry weight were reduced for all collections growing in the elevated pH substrate except for total shoot dry weight for *Q. buckleyi* (Table 2.3). After 109 d, leaf SPAD readings revealed *Q. palustris* (ICT and KSU) began to develop IFC symptoms in the elevated pH substrate while *Q. buckleyi* and *Q. sinuata* var. *breviloba* had not (Figures 2.2A and 2.2B). After 131 d final SPAD values for *Q. palustris* were even lower than from 109 d in elevated pH substrate (19.02 ICT and 16.330 KSU, respectively) (Figures 2.2A and 2.2B). The most recently matured set of leaves of *Q. palustris* seedlings were visually chlorotic after 131 d (Figure 2.3A). *Q. buckleyi* and *Q. sinuata* var. *breviloba* seedlings maintained similar leaf SPAD values in the elevated pH substrate respective to their controls, and neither species developed IFC (Figures 2.2C, 2.2D, 2.3C, and 2.3D respectively).

The concentration of leaf chlorophylls a and b for *Q. palustris* (ICT and KSU) significantly decreased by 57% and 62%, respectively, in the elevated pH substrate (13.11 $\mu g \cdot cm^2$ and 10.74 $\mu g \cdot cm^2$, ICT and KSU, respectively) compared to their controls (30.47 $\mu g \cdot cm^2$ and 28.20 $\mu g \cdot cm^2$, ICT and KSU, respectively) (Figure 2.4). However, the elevated pH substrate did not affect *Q. buckleyi* and *Q. sinuata* var. *breviloba* leaf chlorophyll concentrations (28.46 $\mu g \cdot cm^2$ and 29.25 $\mu g \cdot cm^2$, respectively) which were similar to the controls (30.45 $\mu g \cdot cm^2$ and 28.85 $\mu g \cdot cm^2$, respectively].

Leaf N per kg of leaf dry weight was reduced in *Q. buckleyi* growing in the elevated pH substrate by 16% compared to its control (Table 2.4). No other statistically significant reductions

for leaf N were found within a collection between substrate treatments. Leaf concentration of P per kg of leaf dry weight was reduced in both *Q. buckleyi* and *Q. sinuata* var. *breviloba* by 51% and 43%, respectively, in the elevated pH substrate (Table 2.4). Interestingly, *Q. palustris* ICT and KSU had increases in total leaf P (26% and 21%, respectively), K (75% and 63%, respectively), and Mg (56% and 33%, respectively) per kg of leaf dry weight (Table 2.4). Increases were also observed for leaf Ca content per kg of leaf dry weight in *Q. palustris* (ICT and KSU) and *Q. buckleyi* by 40%, 47%, and 49%, respectively, in the elevated pH substrate treatments, while *Q. sinuata* var. *breviloba* was not affected (Table 2.4).

Total leaf Fe content was significantly reduced for all collections in the elevated pH substrate (Table 2.2 and 2.5). *Q. palustris* (ICT and KSU) experienced reductions of total leaf Fe by 32% and 31%, respectively in the elevated pH substrate, whereas the reduction of leaf Fe in *Q. buckleyi* and *Q. sinuata* var. *breviloba* was 56% and 35%, respectively. When sources were compared within the control substrate treatment, *Q. buckleyi* sequestered more leaf Fe than *Q. palustris* (ICT and KSU) (both $P = 0.0004$) and *Q. sinuata* var. *breviloba* ($P = 0.0352$). *Q. sinuata* var. *breviloba* sequestered more leaf Fe than both ($P = 0.0438$, ICT and $P = 0.0467$, KSU). When sources were compared within the elevated substrate treatment, *Q. buckleyi* sequestered more leaf Fe than *Q. palustris* (ICT and KSU) (both $P < 0.0001$) but not *Q. sinuata* var. *breviloba* ($P = 0.3306$). Also, *Q. sinuata* var. *breviloba* sequestered more leaf Fe *Q. palustris* (ICT and KSU) (both $P < 0.0001$). *Q. buckleyi* and *Q. sinuata* var. *breviloba* maintained similar leaf P:Fe (%:%) between control and elevated pH substrate treatments [(12:1, control and 13:1, elevated; $P = 0.1945$) and (22:1, control and 18:1, elevated; $P = 0.1884$), respectively] while increases in leaf P:Fe were observed for *Q. palustris* ICT and KSU [(23:1, control and 45:1, elevated; $P = 0.0007$) and (22:1, control and 40:1, elevated; $P = 0.0002$),

respectively]. *Q. palustris* (ICT and KSU) also had greater P:Fe compared to *Q. buckleyi* and *Q. sinuata* var. *breviloba* in the elevated pH substrate (all comparisons $P < 0.0001$). All collections sequestered less total leaf Cu when grown in the elevated pH substrate (Table 2.5). Total leaf Mn content was unaffected in *Q. palustris* (ICT), but increased in *Q. palustris* (KSU) and decreased for *Q. buckleyi* and *Q. sinuata* var. *breviloba* in the elevated pH substrate (Table 2.5). We observed varying Mn:Fe ratios ($mg \cdot kg^{-1}$:$mg \cdot kg^{-1}$) for the different collections in the control [(18:1, *Q. palustris* KSU), (17:1, *Q. palustris* ICT), (12:1, *Q. sinuata* var. *breviloba*), and (8:1, *Q. buckleyi*)] and elevated pH substrate [(46:1, *Q. palustris* KSU), (15:1, *Q. palustris* ICT), (7:1, *Q. sinuata* var. *breviloba*), and (6:1, *Q. buckleyi*)]. Mn:Fe for *Q. buckleyi* were consistently less than ratios observed for *Q. palustris* (ICT and KSU) in the control ($P = 0.0016$ and $P = 0.0005$) and in the elevated pH treatment ($P < 0.0001$ and $P = 0.0381$). In addition, *Q. palustris* ICT and KSU sequestered 26% and 49%, respectively; more leaf Zn in elevated pH substrate compared to their controls (Table 2.5). *Q. buckleyi* and *Q. sinuata* var. *breviloba* showed the opposite; reductions in leaf Zn by 67% and 52%, respectively, and less leaf Zn accumulation compared *Q. palustris* ICT and KSU in the elevated pH substrate (Table 2.5).

Expt. 1 2017

Initial substrate leachate pH for both treatments was high and the elevated substrate pH (mean pH = 7.40 over 166 days) remained high for the course of the experiment (Figure 2.5A). Over 166 d, the control substrate pH (mean pH = 6.13) dynamically increased and decreased between applications of aluminum sulfate drench applications. Similar to 2016, there was a significant difference in mean final leachate pH between substrate treatments [(4.00, control and 7.17, elevated), ($P < 0.001$)]. In addition, substrate leachate EC values for the two substrate treatments greatly increased over 166 d. Unlike 2016, final leachate EC was significantly greater

in the control pH substrate [(6.36, control and 5.52 $mS \cdot cm^{-1}$, elevated), (P = 0.0220)] (Figure 2.5B).

Only *Q. sinuata* var. *breviloba* plant height was unaffected by the elevated pH substrate (Tables 2.2 and 2.3). There was a reduction in plant height for both *Q. palustris* (29%) and *Q. buckleyi* (22%) grown in the elevated pH substrate. Stem diameter was reduced for *Q. palustris* (28%) and *Q. sinuata* var. *breviloba* (34%) that were grown in the elevated substrate pH, but unaffected for *Q buckleyi*. Total leaf dry weight of *Q. buckleyi* was not affected by the elevated pH substrate. However, a reduction of leaf dry weight was detected for *Q. palustris* (57%) and *Q. sinuata* var. *breviloba* (45%). A reduction in total leaf area was observed only for *Q. palustris* (50%) grown in the elevated pH substrate (Table 2.3).

Like 2016, leaf SPAD measurements tracked IFC development. After 127 d, *Q. palustris* seedlings had reduced leaf SPAD values (Figure 2.6A). Over the next 32 d, leaf SPAD values for *Q. palustris* slightly decreased in the elevated pH substrate, and its most recently mature foliage remained chlorotic (Figures 2.6A and 2.7). *Q. buckleyi* maintained high final leaf SPAD values in the elevated pH substrate respective to its control and showed no IFC symptoms (Figures 2.6B and 2.8). A slight difference in final leaf SPAD values were observed between substrate treatments for *Q. sinuata* var. *breviloba* (38.21, control and 34.74, elevated; *P* = 0.0380), but all leaves in both treatments remained symptomless of IFC and no visual differences in leaf color were discernable (Figures 2.6C and 2.9).

The most recently matured set of leaves of *Q. palustris* seedlings in the elevated pH substrate showed dramatic IFC symptoms upon termination (Figure 2.7). Once again, *Q. buckleyi* and *Q. sinuata* var. *breviloba* did not show IFC symptoms (Figures 2.8 and 2.9). A large decrease in leaf chlorophyll a and b concentrations (82%) were observed for *Q. palustris* in

elevated pH substrate (36.14 μg·cm^{-2}, control and 6.361 μg·cm^{-2}, elevated) (Figure 2.10). *Q. buckleyi* maintained similar leaf chlorophyll concentration between substrate treatments [(35.83 μg·cm^{-2} control, 35.96 μg·cm^{-2}, elevated) (Figure 2.10). Furthermore, no difference in leaf chlorophyll concentration was observed between substrate treatments for *Q. sinuata* var. *breviloba*; [(36.62 μg·cm^{-2}, control and 34.33 μg·cm^{-2}, elevated).

In 2017, leaf N per kg of dry weight was not affected by the substrate pH treatment for any of the three species (Tables 2.2 and 2.4). Once again, an increase in total leaf P content per kg of dry weight by 30% was observed for *Q. palustris* when grown in the elevated pH substrate. Unlike 2016, leaf P per kg of dry weight of *Q. buckleyi* and *Q. sinuata* var. *breviloba* were unaffected in the elevated pH substrates. Mirroring 2016, increases in leaf K and Mg per kg of dry weight occurred for *Q. palustris* in the elevated pH substrate treatment while both nutrients were similar in both treatments for *Q. sinuata* var. *breviloba*. There was no effect of substrate pH treatment for K content per kg of dry weight in *Q. buckleyi*; however, Mg content per kg of dry weight was decreased at the elevated pH substrate. Total leaf Ca content per kg of dry weight was not influenced by substrate pH treatment in any species (Table 2.4).

P values and micronutrient concentrations from leaves are shown in Tables 2.2 and 2.5, respectively. *Q. palustris* and *Q. sinuata* var. *breviloba* experienced reductions in total leaf Fe by 66% and 31%, respectively, in the elevated pH substrate (Table 2.5). No difference in leaf Fe concentration was observed between substrate treatments for *Q. buckleyi*. Furthermore, no difference in leaf Fe concentration was observed between collections within the control substrate treatment ($P = 0.2997$). When sources were compared within the elevated substrate treatment, *Q. buckleyi* sequestered more leaf Fe than *Q. palustris* ($P < 0.0001$), and *Q. sinuata* var. *breviloba* ($P = 0.0001$). Also, *Q. sinuata* var. *breviloba* sequestered more leaf Fe *Q. palustris* (both $P <$

0.0001). Similar to 2016, *Q. buckleyi* and *Q. sinuata* var. *breviloba* leaves maintained similar P:Fe between substrate treatments [(25:1, control and 29:1, elevated; $P = 0.4560$) and (36:1, control and 65:1, elevated; $P = 0.1134$), respectively] while an increase in leaf P:Fe was observed for *Q. palustris* in the elevated pH substrate (37:1, control and 166:1, elevated; $P = 0.0072$). Furthermore, *Q. palustris* had greater P:Fe ratios compared to *Q. buckleyi* and *Q. sinuata* var. *breviloba* in the elevated pH substrate ($P = 0.0002$ and $P = 0.0028$, respectively). Reductions in total leaf Mn concentrations were observed within all collections in the elevated pH substrate [(87%, *Q. palustris*), (88%, *Q. sinuata* var. *breviloba*), and (87%, *Q. buckleyi*)]. Like 2016, we observed varying leaf Mn:Fe values between collections in the control [(61:1, *Q. palustris*), (14:1, *Q. sinuata* var. *breviloba*), and (14:1, *Q. buckleyi*)] and elevated pH substrates [(22:1, *Q. palustris*), (3:1, *Q. sinuata* var. *breviloba*), and (2:1, *Q. buckleyi*)]. Leaf Mn:Fe for *Q. buckleyi* and *Q. sinuata* var. *breviloba* were lower than ratios observed for *Q. palustris* in the control (both $P < 0.0001$) and in the elevated pH treatment ($P = 0.0008$ and $P = 0.0017$, respectively). Additionally, Mn:Fe ratios were reduced within all collections in elevated pH substrates compared to their controls ($P = 0.0007$, *Q. palustris*; $P = 0.0006$, *Q. sinuata* var. *breviloba*; and $P = 0.0013$, *Q. buckleyi*). Similar to 2016, all collections experienced reductions in total leaf Cu when grown in the elevated pH substrate compared to the controls (Table 2.5). Additionally, *Q. buckleyi* and *Q. sinuata* var. *breviloba* seedlings experienced reductions in total leaf Zn in the elevated pH, while, leaf Zn for *Q. palustris* was not affected (Table 2.5).

Expt. 2 (2016 to 2017)

In the 2016 growing season, substrate leachate pH for all treatments began at similar values, and only after 90 d did the substrate treatment pH began to distinctly separate (Figure 2.11A). At the start of the 2017 growing season, leachate pH for the control pH substrate began

higher than expected (pH = 7.49) (Figure 2.11A). Similar to Expt. 1 2017, pH-lowering aluminum sulfate drenches were used to reduce the pH in control substrates which created a dynamic trend in leachate pH (Figure 2.11C). After 166 d, there was a difference in final leachate pH values between substrate treatments ($P < 0.001$). Mean leachate pH for the control, low, medium, and high pH substrates over the last 102 d was 4.91, 6.79, 7.24, and 7.38, respectively. Substrate leachate EC for all pH substrates in 2016 and 2017 increased approximately three-fold over 132 d and 166 d respectively (Figures 2.11B and 2.11D). At the end of the growing season in 2017, a difference in final leachate EC between substrate treatments was observed ($P < 0.0041$). The control pH substrate, receiving the aluminum sulfate drenches, had the highest EC values (7.08 $mS \cdot cm^{-1}$).

Intermediate responses in plant growth characteristics were observed after 171 d for *Q. palustris*, while growth characteristics for *Q. buckleyi* and *Q. sinuata* var. *breviloba* were not affected by substrate pH treatments. (Tables 2.6 and 2.7). Height and the number of leaves produced were not affected by substrate treatment for any collection, and only stem diameter of *Q. palustris* KSU was reduced in the medium and high pH substrate treatments. Furthermore total leaf area was reduced for *Q. palustris* ICT in the high compared to the control and low pH substrate treatments. Total leaf and shoot dry weights for *Q. palustris* were reduced in the high pH substrate [(44% and 34%, ICT) and (30% and 37%, KSU), respectively] (Table 2.7).

Over the 2016 growing season, $CaCO_3$ drenches for the low, medium, and high pH substrates did not produce discernable differences in leaf SPAD values (Figure 2.12A), but after 133 d in the 2017 growing season, *Q. palustris* (ICT and KSU) in the medium and high pH substrates had lower leaf SPAD values compared to the low and control pH substrates (Figure 2.13A, 2.13B and 10). After 168 d in 2017, *Q. palustris* had low leaf SPAD values in the

medium (24.05, ICT and 20.72, KSU) and high pH substrates (15.01, ICT and 14.99, KSU) with visual IFC symptoms (Figure 2.13A, 2.13B and 2.14). *Q. buckleyi* and *Q. sinuata* var. *breviloba* maintained high leaf SPAD values and green leaf color in all substrate pH treatments throughout the growing season (Figure 2.13C, 2.13D, 2.15, and 2.16).

Similarly, large reductions in leaf chlorophyll concentration were observed in *Q. palustris* in the medium and high pH substrates (ICT: 10.67 $\mu g \cdot cm^{-2}$ and 5.474 $\mu g \cdot cm^{-2}$, respectively) and (KSU: 11.36 $\mu g \cdot cm^{-2}$ and 8.89 $\mu g \cdot cm^{-2}$, respectively) compared to the controls (ICT: 24.50 $\mu g \cdot cm^{-2}$ and KSU: 25.24 $\mu g \cdot cm^{-2}$; all comparisons $P < 0.0001$) (Figure 2.17). *Q. palustris* in the low pH substrate did not have statistically lower leaf chlorophyll concentrations compared to the controls (ICT: 23.62 $\mu g \cdot cm^{-2}$, $P = 0.3470$) and KSU: 23.13 $\mu g \cdot cm^{-2}$, $P = 0.4406$). Unlike *Q. palustris* (ICT and KSU), *Q. buckleyi* and *Q. sinuata* var. *breviloba* trees maintained high leaf chlorophyll concentrations in the low, medium, and high substrate treatments [(*Q. buckleyi*: 25.13 $\mu g \cdot cm^{-2}$, 25.45 $\mu g \cdot cm^{-2}$, and 24.28 $\mu g \cdot cm^{-2}$ and *Q. sinuata* var. *breviloba*: 25. $\mu g \cdot cm^{-2}$, 25.32 $\mu g \cdot cm^{-2}$, and 24.80 $\mu g \cdot cm^{-2}$) respectively] compared to the controls (*Q. buckleyi*: 25.55 $\mu g \cdot cm^{-2}$ and *Q. sinuata* var. *breviloba*: 23.32 $\mu g \cdot cm^{-2}$). Table 2.6 contains *P* values from a one-way analysis variance for comparison of pH substrate treatments within a collection, and Figure 2.17 displays groupings from a two-way analysis variance since the interaction between main effects was significant ($P < 0.0001$).

No differences in leaf N per kg of dry weight were observed within a collection between substrate treatments, but *Q. palustris* (ICT and KSU) experienced less total leaf C per kg of dry weight in the low, medium, and high pH substrates (Tables 2.6 and 2.8). As in Expt. 1, there was an increase in leaf P per kg of dry weight in *Q. palustris* (ICT and KSU) as substrate pH increased, which was not observed for *Q. sinuata* var. *breviloba* or *Q. buckleyi* (Table 2.8).

Extreme increases in total leaf K contents per kg of dry weight were observed for *Q. palustris* (ICT and KSU) in the medium and high pH treatment, while, leaf K content per kg of dry weight for *Q. buckleyi*, and *Q. sinuata* var. *breviloba* was unaffected between substrate treatments (Table 2.8). A general increase of leaf Ca per kg of dry weight was detected in *Q. palustris* (ICT and KSU) and *Q. buckleyi* growing in the medium and high pH substrates. There was no effect of substrate pH treatment on leaf Mg per kg of dry weight for any plant collection (Table 2.8).

Q. palustris KSU experienced reductions in leaf Fe in the high pH substrate compared to the control and low pH substrates (Table 2.9). Leaf Fe content for *Q. palustris* ICT was unaffected between the substrate treatments, but when individual comparisons were made, statistical differences for leaf Fe were observed between the low and medium ($P = 0.0388$) and medium and high pH substrates ($P = 0.0274$). Leaf Fe content for *Q. palustris* ICT was not reduced in the high compared to the control or low pH substrates ($P = 0.8370$ and $P = 0.9659$, respectively). Leaf Fe contents for *Q. buckleyi* and *Q. sinuata* var. *breviloba* between substrate pH treatments were not statistically affected within the collection. No difference in leaf Fe concentration was observed between collections within the control or substrate treatments ($P = 0.1843$ and $P = 0.2317$, respectively). When sources were compared within the medium pH substrate treatment, *Q. buckleyi* sequestered more leaf Fe than *Q. palustris* (ICT: $P = 0.0062$ and KSU: $P = 0.0039$), but not *Q. sinuata* var. *breviloba* ($P = 0.7578$). Also, *Q. sinuata* var. *breviloba* sequestered more leaf Fe *Q. palustris* (ICT: $P = 0.0071$ and KSU: $P = 0.0111$). In the elevated pH substrate, *Q. buckleyi*, *Q. sinuata* var. *breviloba*, and *Q. palustris* ICT sequestered more leaf Fe than *Q. palustris* KSU ($P = 0.0006$, $P = 0.0114$, and $P = 0.005$, respectively). Neither *Q. buckleyi* nor *Q. sinuata* var. *breviloba* sequestered greater leaf Fe than *Q. palustris* ICT ($P = 0.2674$ and $P = 0.6665$, respectively). Like the previous two experiments, each Texas

native maintained similar P:Fe between the different substrate treatments [(*Q. buckleyi*: 21:1, 23:1, 23:1, and 19:1; $P = 0.6497$) and (*Q. sinuata* var. *breviloba*: 43:1, 43:1, 36:1, and 35:1; $P = 0.6042$,)]. Increases in leaf P:Fe were observed for *Q. palustris* KSU collections in the medium and elevated compared to the control pH substrates [(22:1, control), (84:1, medium; $P < 0.0054$), and (114, high; $P < 0.0003$)]. Furthermore, Increases in leaf P:Fe occurred for *Q. palustris* ICT between the control and medium pH substrates (33:1, control and 69:1, medium; $P < 0.0114$) but not high because of the abnormal trend in its total leaf Fe content. Leaf Mn concentration was reduced in all collections as substrate pH increased (Table 2.9). Reductions in Mn:Fe occurred within each collection in their medium and elevated pH substrates compared to the controls (data not shown). *Q. palustris* (ICT and KSU) and *Q. buckleyi* experienced a general reduction in total leaf Cu as substrate pH increased (Table 2.9). Additionally, leaf Cu content was only reduced for *Q. sinuata* var. *breviloba* in the medium and high compared control and low pH substrates. Leaf Zn contents were unaffected between pH treatments for *Q. palustris* (ICT and KSU), while leaf Zn was depressed for *Q. buckleyi*, and *Q. sinuata* var. *breviloba* in the medium and high pH substrates compared to the respective controls (Table 2.9).

Discussion and Conclusions

We investigated the IFC development of containerized *Q. palustris*, *Q. buckleyi*, and *Q. sinuata* var. *breviloba* seedlings growing in elevated pH substrate to evaluate their potential adaptability to high soil pH. *Q. palustris*, a popular landscape taxon is known to develop Fe-induced IFC when grown in high pH and calcareous soils. However, there is speculation that *Q. buckleyi*, and *Q. sinuata* var. *breviloba* do not develop IFC in high pH soils, because some provenances of these taxa grow in soils derived from limestone and are tolerant to high pH soils (Neely, 1976; Sternberg and Wilson, 1995). Like others, we utilized indirect (leaf SPAD

measurements) and direct methods (leaf chlorophyll a and b extraction) to determine if these species developed IFC and, if so, to what extent were leaf nutrition and growth characteristics impacted (Denig et al., 2014; Hsieh and Waters, 2016; McNamara and Pellet, 2001). Generally, leaf SPAD values measure some proportionality to leaf chlorophyll concentration, but for this study we did not create a regressions for each collection being evaluated (Bielinis, and Robakowski, 2015; Gáborčík, 2003).

Collections of *Q. palustris* developed IFC and had considerably reduced leaf SPAD values and low chlorophyll a and b concentrations when grown in the elevated pH substrates. We observed a distinct separation in ranges for mean SPAD and leaf chlorophyll between experiments for chlorotic and non-chlorotic *Q. palustris* seedlings upon experiment termination. *Q. palustris* displaying symptoms of IFC had SPAD values that ranged from 14 to 18.9, whereas symptomless trees SPAD values ranged from 36.7 to 38.7. These findings are consistent with Berrang and Steiner (1980) where leaf SPAD measurements for containerized *Q. palustris* seedlings decreased when grown at high pH Similarly, the chlorophyll concentration in *Q. palustris* with IFC ranged from 6.36 $\mu g \cdot cm^{-2}$ to 11.9 $\mu g \cdot cm^{-2}$ while for symptomless trees chlorophyll concentration ranged from 24.2 $\mu g \cdot cm^{-2}$ to 36.1 $\mu g \cdot cm^{-2}$. Unlike *Q. palustris*, collections of *Q. buckleyi* from and *Q. sinuata* var. *breviloba* did not develop IFC when grown in any of the pH substrate treatments. Sustained leaf SPAD values and chlorophyll concentrations for *Q. buckleyi* and *Q. sinuata* var. *breviloba* seedlings in elevated pH substrates may indicate these two species have a greater tolerance to high soil pH and thus a better ability to resist IFC development.

Decreases in total leaf Fe content were observed when *Q. palustris* was grown in an elevated pH substrate (except *Q. palustris* ICT receiving 4.77% $CaCO_3$ in Expt. 2). These

findings coincide with Hauer and Dawson (1996) when *Q. palustris* seedlings where grown in an alkaline (p H = 7.5) compared to an acidic (pH = 5.5) sterilized soil-based medias (40.7 $mg \cdot kg^{-1}$ to 51.3 $mg \cdot kg^{-1}$ Fe, alkaline and 54.1 $mg \cdot kg^{-1}$ to 60.0 $mg \cdot kg^{-1}$ Fe, acidic). Reports of total leaf Fe content for healthy *Q. palustris* from field research plots to range from 35.0 $mg \cdot kg^{-1}$ to 68.0 $mg \cdot kg^{-1}$ (Mills and Benton, 1996). We observed overlapping ranges for mean total leaf Fe content for between chlorotic and non-chlorotic *Q. palustris* seedlings over the three experiments (12.8 $mg \cdot kg^{-1}$ to 45.5 $mg \cdot kg^{-1}$, chlorotic and 35.3 $mg \cdot kg^{-1}$ to 64.9 $mg \cdot kg^{-1}$, non- chlorotic). The earliest pioneers in plant nutrition recognized for chlorophyll formation to occur, total leaf Fe content must exceed a threshold predetermined by a species and its growing conditions (Jacobson, 1945). In addition, total leaf Fe on a dry weight basis, does not always correlate with the amount of leaf chlorophyll, because total Fe is not indicative to amount of physiologically active Fe (Nikolic and Römheld, 2002). In Expt. 2, it was puzzling that *Q. palustris* ICT receiving 4.77% $CaCO_3$ had a high leaf Fe content yet had extremely low leaf chlorophyll content than its control. The occurrence of IFC symptoms in leaves with sufficient Fe concentration has been termed ‘the chlorosis paradox’, and it has been observed and described in grape (*Vitus* L.), peach (*Prunus* L.), and more recently citrus (*Citrus* L.) (Martinez-Cuena and Primo-Capella, 2017; Morales et al., 1998; Nikolic and Römheld, 2002; Römheld, 2008). ‘The chlorosis paradox’ has been described as the occurrence of normal yet immobilized total concentrations of Fe in chlorotic leaves that are restricted from reaching morphological maturity (Nikolic and Römheld, 2002). This occurs from inadequate ferric Fe reduction before entry into the leaf symplast, because FCR expression and activity are low in chlorotic and morphologically stunted leaves making Fe difficult to acquire from the apopast (Martinez-Cuena and Primo-Capella, 2017; Nikolic and Römheld, 2008). Furthermore, even though the leaf area, shape, and

size was not documented on the set of leaves from which leaf chlorophylls and nutrient analysis were taken in our study, some leaves were observed to be morphologically stunted in size. This would be the first occurrence of the 'the chlorosis paradox' in a species of *Quercus* to our knowledge. Another explanation of a high total Fe content from chlorotic leaves could have simply been caused by human or sample contamination. A common leaf tissue contaminant is soil Fe. When plant tissues are contaminated by soil Fe, laboratory analyses are generally observed above 300 $mg \cdot kg^{-1}$; this would suggest that the unusually high total Fe concentration we observed from chlorotic leaves was not caused by soil Fe contamination since the greatest sample from the chlorotic tissue had no more than 63 $mg \cdot kg^{-1}$ total Fe (Mayland and Sneva, 1983).

Total leaf Fe content varied for *Q. buckleyi* and *Q. sinuata* var. *breviloba* seedlings. In some cases the Fe concentration was reduced when these species were growing in an elevated pH substrate. However, no manifestation of IFC developed, and both of these species generally maintained higher total leaf Fe than *Q. palustris* as substrate pH increased. This would suggest that *Q. buckleyi* and *Q. sinuata* var. *breviloba* are more Fe efficient in sequestering greater amounts of substrate Fe compared to *Q. palustris* from elevated pH substrates. This has been observed in other tree species natively distributed on high pH soils that have greater activities of the rhizosphere acidification and FCR by their fine root tissues (Marschner, 2011 and Venturas et al., 2014). We observed a large variation for mean ranges of total leaf Fe content of non-chlorotic *Q. buckleyi* and *Q. sinuata* var. *breviloba* seedlings (41.7 $mg \cdot kg^{-1}$ to 208 $mg \cdot kg^{-1}$ Fe in *Q. buckleyi* and 27.4 $mg \cdot kg^{-1}$ to 134 $mg \cdot kg^{-1}$ Fe in *Q. sinuata* var. *breviloba*). While previous research has not indicated a working range for total Fe from healthy leaves for *Q. buckleyi* and for *Q. sinuata* var. *breviloba*, total leaf Fe content greatly varies for similar species when grown

in field production [38.0 mg·kg^{-1} to 126 mg·kg^{-1} Fe, white oak (*Quercus alba* L.) and 40.0 mg·kg^{-1} to 61.0 mg·kg^{-1} Fe , *Q. shumardii*] (Mills and Benton, 1996). For the control pH substrates in 2017, aluminum sulfate drenches were applied to reduce substrate pH. Even though excess aluminum may interfere with the translocation of Fe, Mn, and Zn from roots to shoots and cause Fe deficiency at a low pH in agronomic crops, it has not shown to detrimentally affect leaf chlorophyll concentration and photosynthetic capacity in Japanese blue oak (*Quercus glauca* Thumb.) (Akaya Takenaka, 2001; Bityutskii et al., 2017; Schmitt et al., 2016). Although less total leaf Fe sequestration occurred in the 2017 substrates, no IFC was observed in the control pH substrate treatments suggesting leaf chlorophyll formation was unimpeded (Kobayashi and Nishizawa, 2012; Marschner, 2011; Martinez- Cuena and Primo- Capella, 2017; Waters and Troupe 2012). This would further support the assumption that total Fe content in leaves is not the only factor contributing to IFC (Nikolic and Römheld, 2002).

The elevated pH substrate caused other micronutrient imbalances in the leaves of seedlings. Total leaf Mn and Cu were consistently reduced in the elevated pH substrates within *Q. palustris*, *Q. buckleyi* and *Q. sinuata* var. *breviloba*. Chlorotic *Q. palustris* seedlings growing in the elevated pH substrates had equal or greater leaf Zn compared to non-chlorotic *Q. buckleyi* and *Q. sinuata* var. *breviloba* seedlings growing in the elevated pH substrates. Messenger (1993 and 1994) suggested Mn and Zn deficiencies are associated with IFC in *Q. palustris*. Even though we did not determine total plant nutrient contents or their availabilities in the substrates, we did not observe a Zn deficiency in the leaves of chlorotic *Q. palustris* seedlings. Our findings would suggest that IFC does not interfere with Zn accumulation in leaves of *Q. palustris*. All Zn concentration in *Q. palustris* in this work was within the previously reported concentrations for healthy leaves of trees from research plots (29 mg·kg^{-1} to 88 mg·kg^{-1}; Mills and Benton, 1996).

Because Zn availability for uptake by the root is normally reduced by high soil pH and $CaCO_3$ content, we speculate that the foliar Zn accumulation is not caused from increased uptake but rather its mobilization from root and shoot tissues into leaves (Iratkar et al., 2014; Rehman et al., 2012). Furthermore, we observed the typical Mn to Fe antagonistic relationship where total leaf Mn was always greater than total leaf Fe (Marschner, 2011). We generally found smaller leaf Mn:Fe ratios in seedlings growing in the elevated pH substrates suggesting total leaf Mn sequestration is more greatly impacted at a higher pH than Fe with the substrates we used. *Q. buckleyi* generally had lower Mn:Fe ratios than *Q. palustris* in elevated pH substrates suggesting that, when Fe is limiting, *Q. buckleyi* may uptake Fe more efficiently over Mn than *Q. palustris*.

While differences in leaf micronutrients occurred between many of the treatments, variations in macronutrient were observed but not outside normal ranges other than total leaf K. The chlorotic *Q. palustris* seedlings had greater total leaf K contents per kg of dry weight than non-chlorotic *Q. palustris* seedlings in all experiments; this trend was not observed for either *Q. buckleyi* or *Q. sinuata* var. *breviloba*. The an observed range for total K content per kg of dry weight in leaves from field research *Q. palustris* trees was from 0.49% to 1.25% (Mills and Benton, 1996). We observed ranges of total leaf K from 1.35% to 2.22% in chlorotic leaves. These findings are consistent with others that found K contents per kg of dry weight were higher in chlorotic leaf samples in *Prunus* L. (Belkhodja et al., 1998; Köseoğlu, 1995). The manifestation of greater K contents from chlorotic leaves could simply be explained by the fact that more leaf tissue is required for sampling, because chlorotic leaves generally had less mass. Or, the influx of excess K in chlorotic leaves was most likely secondary to the manifestation of IFC to possibly to detoxify reactive oxygen species by reducing NADPH oxidase activity and maintaining photosynthetic electron transport, increased activity of the root plasma membrane

ATPases involved in proton excretion, or organic acid accumulation that occurs under Fe deficiency (Cakmak, 2005; Welkie and Miller, 1993). Another explanation would propose that the greater K contents were observed because it takes more chlorotic leaf tissue to acquire the same mass from a healthy leaf. We also observed increases in total leaf P content per kg of dry weight and P:Fe for chlorotic *Q. palustris*, but not healthy *Q. buckleyi* and *Q. sinuata* var. *breviloba* seedlings. P:Fe from healthy leaves of *Q. palustris* has shown to range from 22:1 to 36:1 (Welkie and Miller, 1993). We observed ranges of P:Fe in chlorotic *Q. palustris* leaves (22:1 to 37:1, healthy and 50:1 to 166:1, chlorotic). It has been suggested that as P:Fe increases, inorganic Fe becomes immobilized from its precipitation in leaf tissues and becomes unavailable for chlorophyll production in garden tomato (*Solanum lycopersicum* L.), but in peach, (*Prunus* L) increases of P:Fe have occurred in non-chlorotic leaves supplemented with P (De Kock et al., 1979; Romera et al., 1991). This may suggest that *Q. buckleyi* and *Q. sinuata* var. *breviloba* avoid leaf Fe immobilization by avoiding high leaf P:Fe when Fe is limited in availability.

The growth characteristics of seedlings in the elevated pH substrate varied between experiments. In Expt. 1, growth characteristics for *Q. palustris* seedlings were most negatively impacted by the elevated pH substrate. Stem diameter, total leaf area, and total leaf and shoot dry weights were often reduced. Total leaf dry weights for *Q. buckleyi* in 2016 and for *Q. sinuata* var. *breviloba* had also been consistently reduced in Expt. 1. Effects on growth characteristics was less obvious between treatments in Expt. 2 which could be attributed to difference in initial seedling establishment. In Expt. 1, elevated pH substrate treatments began at planting and those in Expt. 2 did not, thus all seedlings began at a lower base pH and gradually were subjected to a higher pH as time progressed. No single growth characteristic between all three experiments was consistently indicative of susceptibility or tolerance of a collection to IFC, but total leaf area was

the best growth characteristic indicator. When assessed as a whole, the growth characteristics did not consistently support the relative pH tolerances of the collections indicated by leaf SPAD values and leaf chlorophyll concentrations. The inconsistency in grow characteristics is similar to what others have observed when screening progeny of *Q. palustris* from multiple sources and birch (*Betula* L.) for their susceptibility to IFC (McNamara and Pellett, 2001; Tobolski, 1987). Furthermore, we must acknowledge that much variation in growth habit may exist between the collections we used. Progeny of *Quercus* may be extremely heterozygous because they are monoecious species with a protandrous systems which can enhance successful crossing with individuals in the same population and even between different species thus creating greater genetic variation (Konar et al., 2017; Muir et al., 2004; Rocheta et al., 2014).

In summary, there is speculative evidence that indicates that *Q. buckleyi* and *Q. sinuata* var. *breviloba* may be more capable of resisting Fe-induced IFC by sequestering greater amounts of substrate Fe than *Q. palustris* from high pH substrates and having a better use efficiency of Fe by avoiding excessively high leaf P:Fe and Fe:Mn when Fe is limited. Since total leaf Fe is not always indicative of the amount of physiologically active Fe used for chlorophyll formation, further evaluations of these species should be conducted to determine the mechanisms behind their resilient nature to Fe-induced IFC. In addition, field evaluations with considerations of provenance performance in different hardiness zones should be used to determine their potential as a more suitable species for use in landscapes with high pH soils.

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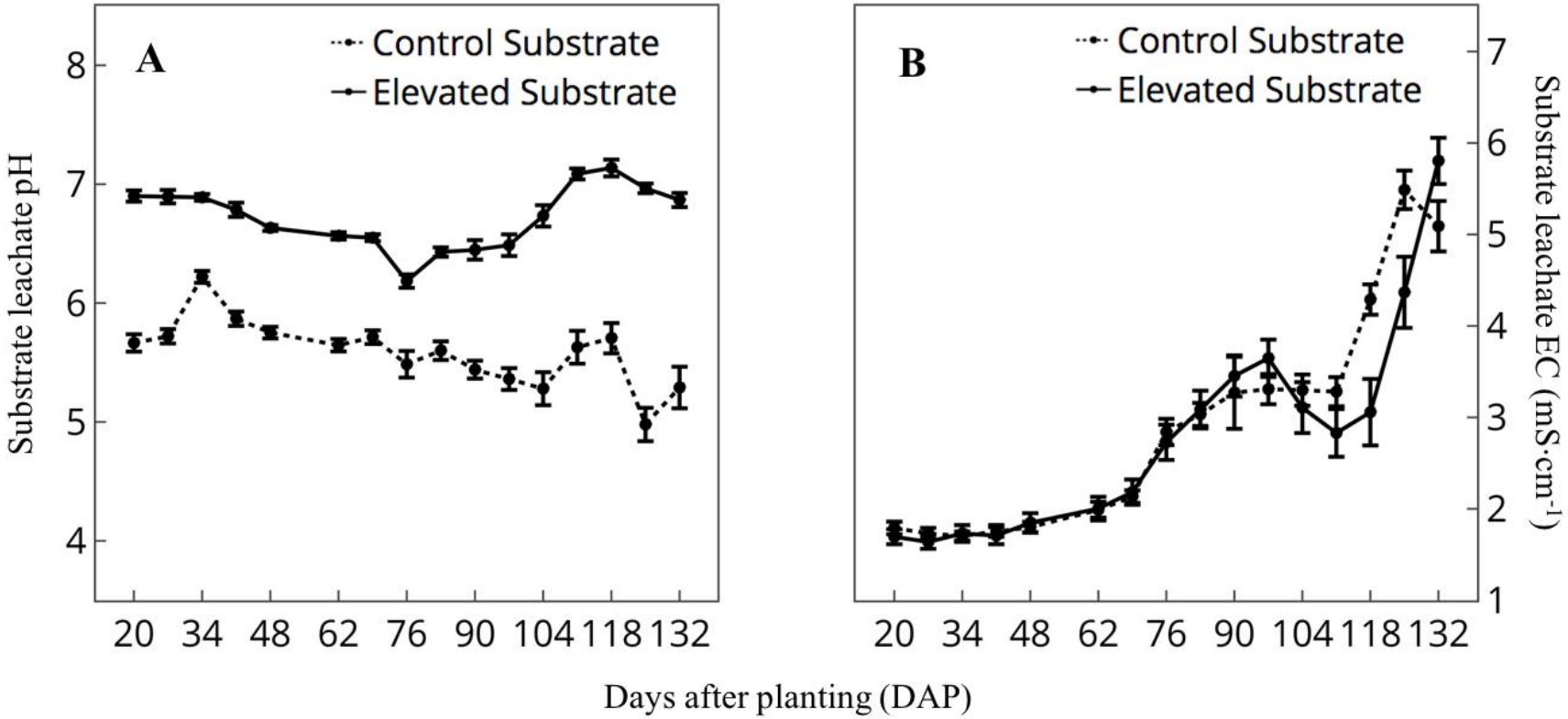

Figure 2.1 Expt. 1 2016: Substrate leachate (A) pH (5.58, control and 6.72, elevated mean pH) and (B) electrical conductivity (EC) trends for substrate treatments in 2016. Substrates began with either 0 kg·m^{-3} (control) or 11.9 kg·m^{-3} dolomitic lime (elevated) and received twice-weekly drenches of either 120 mL 0% (control) or 4.77% flowable $CaCO_3$ (elevated) over the course of 2016. Each point represents in mean ± SE (n = 44) except (n = 42) in the elevated treatment.

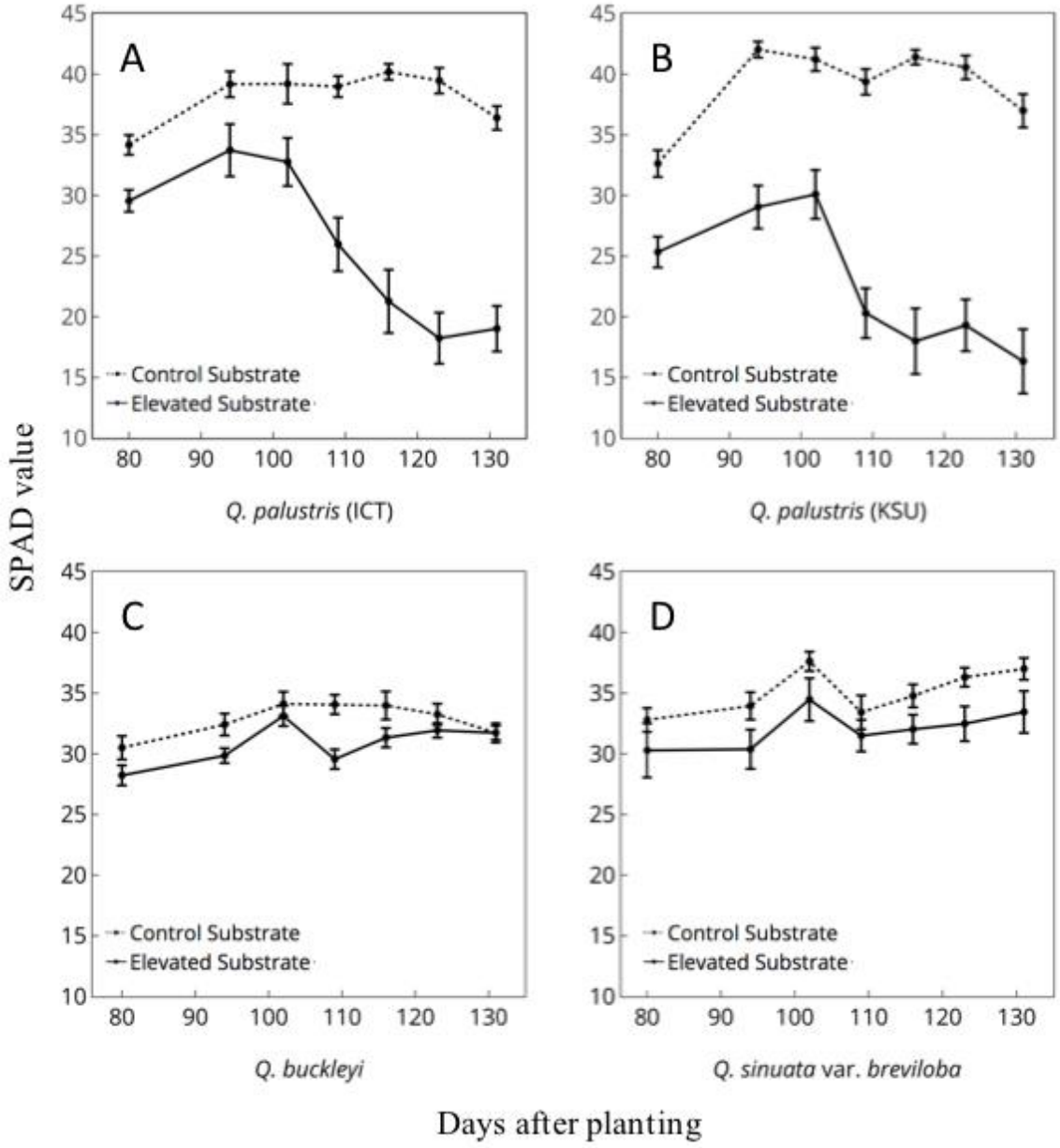

Figure 2.2 Expt. 1 2016: leaf SPAD trends for substrate pH treatments. Substrates began with either 0 kg·m^{-3}, (control, dotted lines) or 11.9 kg·m^{-3} dolomitic lime (elevated, solid lines) and received either 120 mL of 0% (control) or 4.77% flowable $CaCO_3$, elevated twice-weekly over the course of the experiment. (A and B) Leaf SPAD for *Q. palustris* (ICT) and (KSU) respectively; (C) *Q. buckleyi*; and (D) *Q. sinuata* var. *breviloba.* Each point represents a mean ± SE (n = 11), except (D) mean ± SE (n = 9) in the elevated treatment.

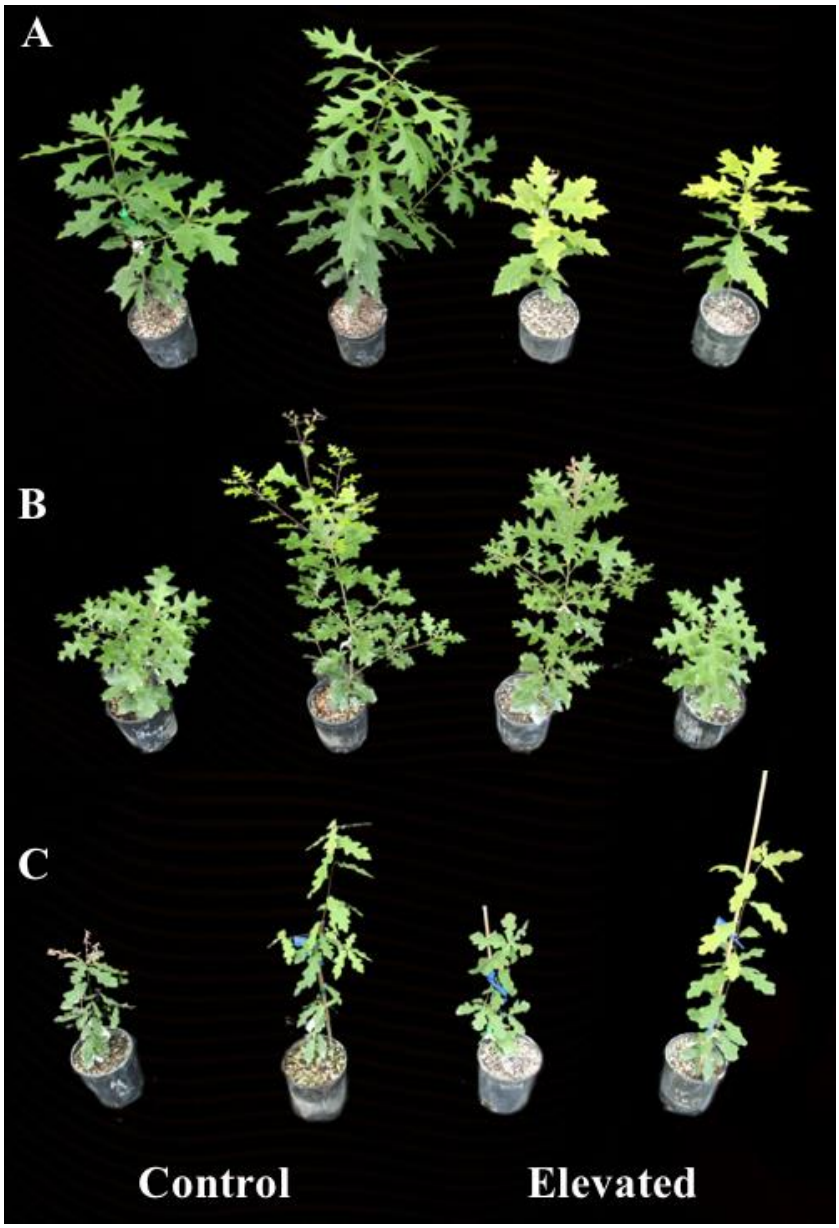

Figure 2.3 Expt. 1 2016: Photographs of 2 replications per treatment taken after 133 d (does not include treatments for *Q. palustris* ICT). From left to right, control (mean pH = 5.58) and elevated (pH = 6.72). Substrates began with either 0 $kg \cdot m^{-3}$ (control) or 11.9 $kg \cdot m^{-3}$ dolomitic lime (elevated) and received twice-weekly drenches of either 120 mL 0% (control) or 4.77% flowable $CaCO_3$ (elevated) over the course of 2016. for (A) *Q. palustris* (KSU); notice stunted growth and IFC in the elevated treatment. (B) *Q. buckleyi* and (C) *Q. sinuata* var. *breviloba.*

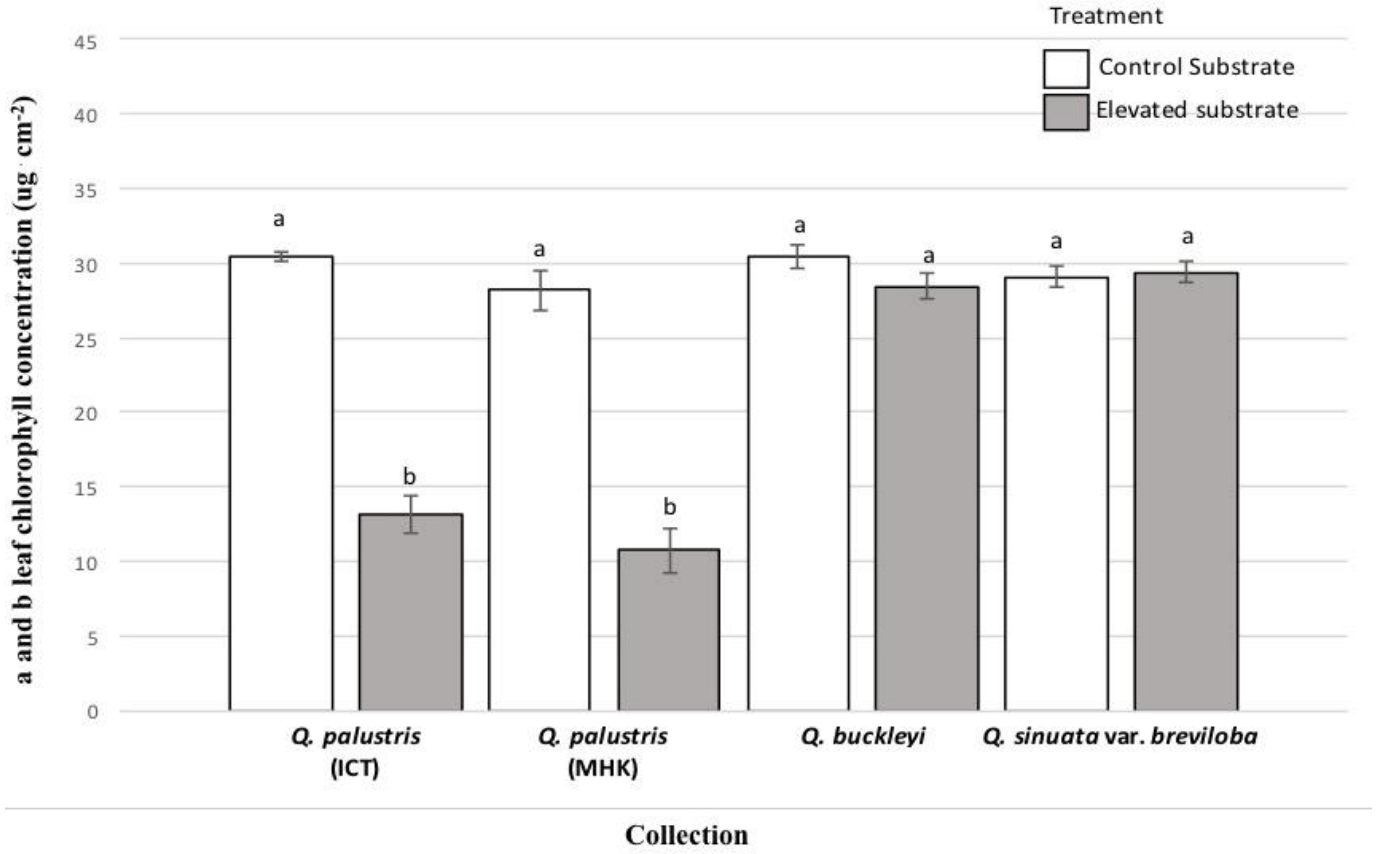

Figure 2.4 Expt. 1 2016: leaf chlorophyll a and b concentrations from leaves after 135 d for *Q. palustris* (ICT and KSU), *Q. buckleyi*, and *Q. sinuata* var. *breviloba* receiving either 120 mL of 0% (control) or 4.77% flowable $CaCO_3$ (elevated) substrate drenches twice-weekly; control (mean pH = 5.58) and elevated (pH = 6.72). Each bar represents in mean ± SE with n = 5 combined reps; expect for *Q. sinuata* var. *breviloba*, elevated = 3 combined reps. Data subjected to analysis of variance using LSM differences were assessed at $P \leq 0.05$ using Proc GLM/lsmeans option with Fisher' s Protected LSD in SAS® University Edition (2018).

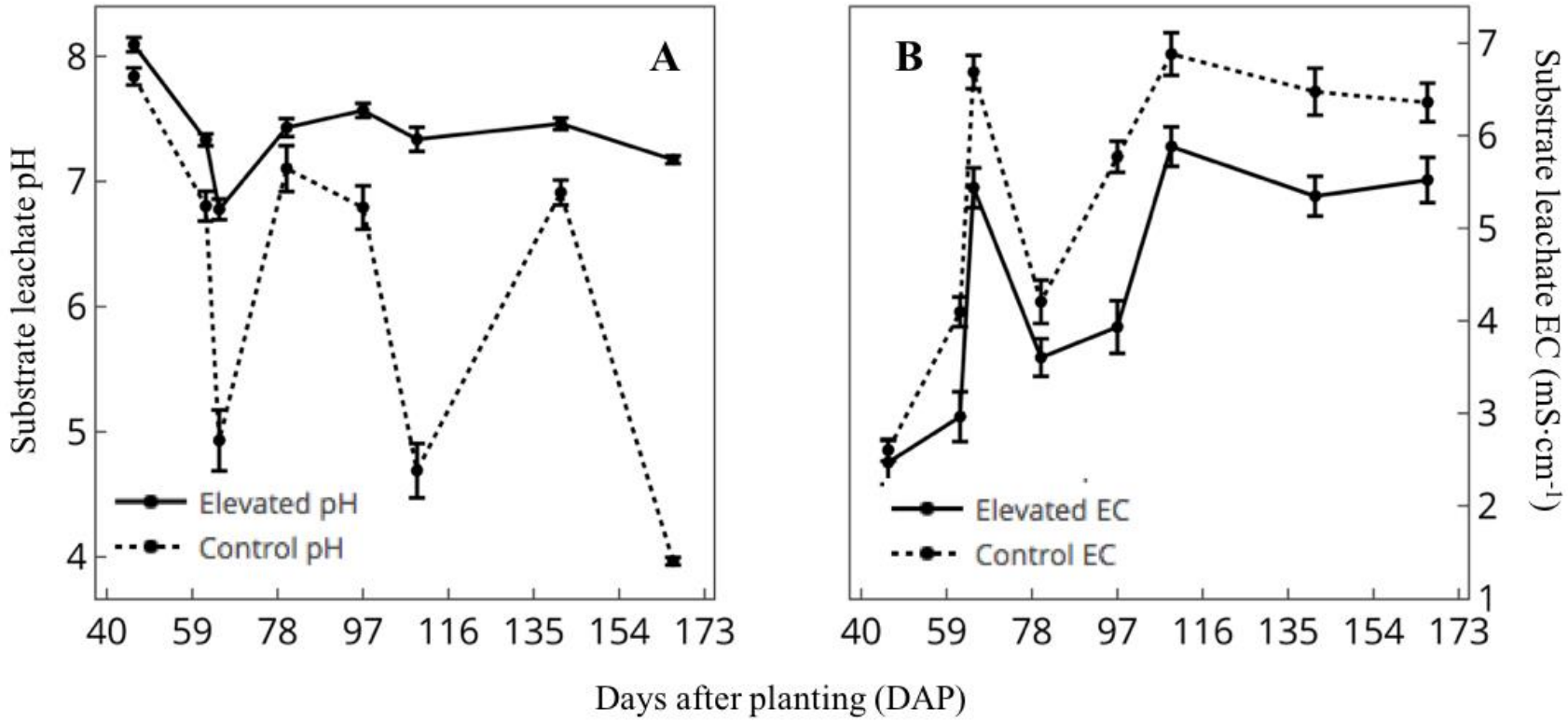

Figure 2.5 Expt. 1 2017: Substrate leachate (A) pH (6.13, control and 7.40, elevated mean pH) and (B) electrical conductivity (EC) trends for substrate treatments in 2017. Substrates began with either 0 $kg \cdot m^{-3}$ (control) or 11.9 $kg \cdot m^{-3}$ dolomitic lime (elevated) and received twice-weekly 120 mL drenches of either 0% (control) or 4.77% flowable $CaCO_3$ (elevated). Each point represents in mean ± SE (n = 37)

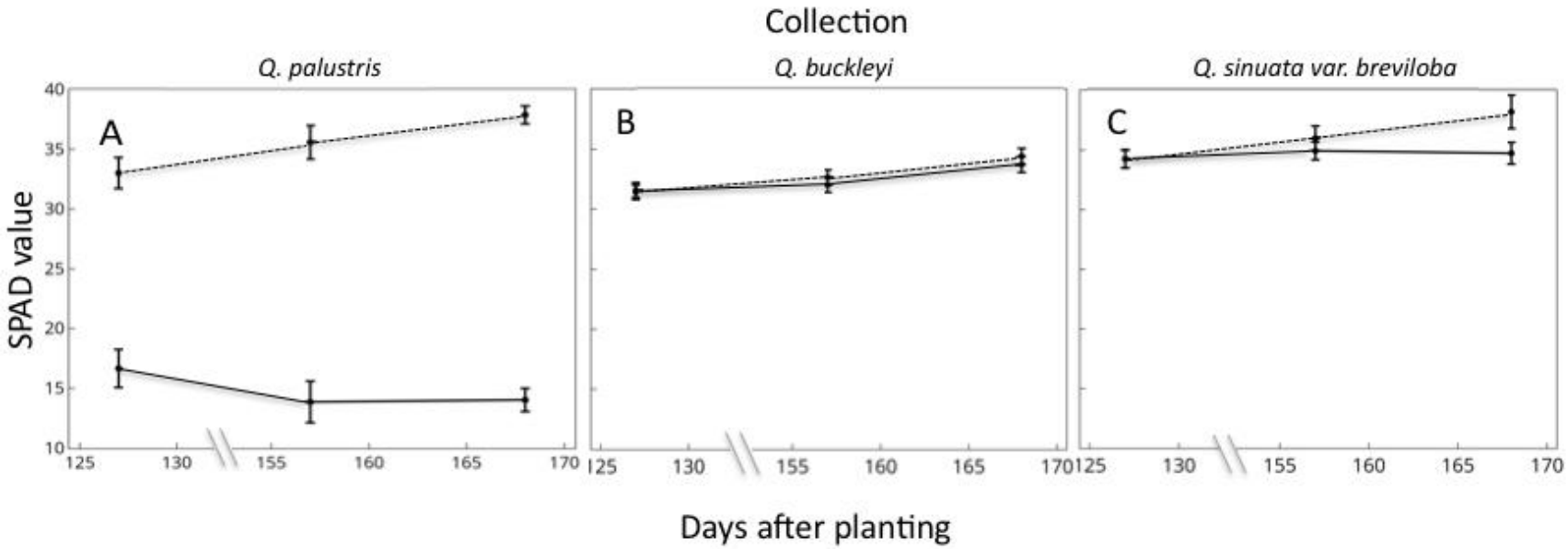

Figure 2.6 Expt. 1 2017: Leaf SPAD trends after 168 d for treatments for substrates that began with either 0 $kg \cdot m^{-3}$ (control, dotted lines) or 11.9 $kg \cdot m^{-3}$ dolomitic lime (elevated, solid lines) and received twice-weekly 120 mL drenches of either 0% (control) or 4.77% flowable $CaCO_3$ (elevated) over the course of the experiment. (A, B, and C) represent mean leaf SPAD for control and elevated pH substrates in *Q. palustris*, *Q. buckleyi*, and *Q. sinuata* var. *breviloba* respectively. Each point represents a mean ± SE (n = 10), except (C) (n = 7).

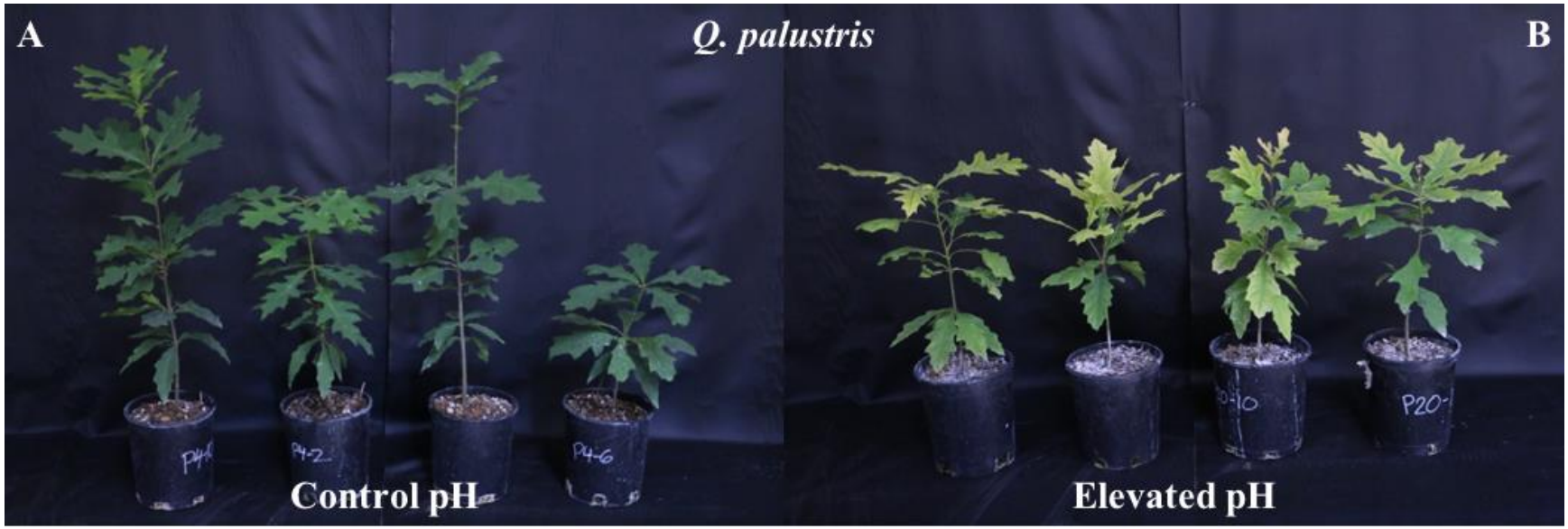

Figure 2.7 Expt. 1 2017: Four representative replications per treatment of *Q. palustris* taken after 172 d. (A) in the control pH substrate (mean pH = 6.13) (B) in the elevated pH substrate (mean pH = 7.40); notice stunted growth and IFC in the elevated treatment. Substrates began with either 0 $kg \cdot m^{-3}$ (control) or 11.9 $kg \cdot m^{-3}$ dolomitic lime (elevated) and received twice-weekly 120 mL drenches of either 0% (control) or 4.77% flowable $CaCO_3$ (elevated) over the course of the experiment.

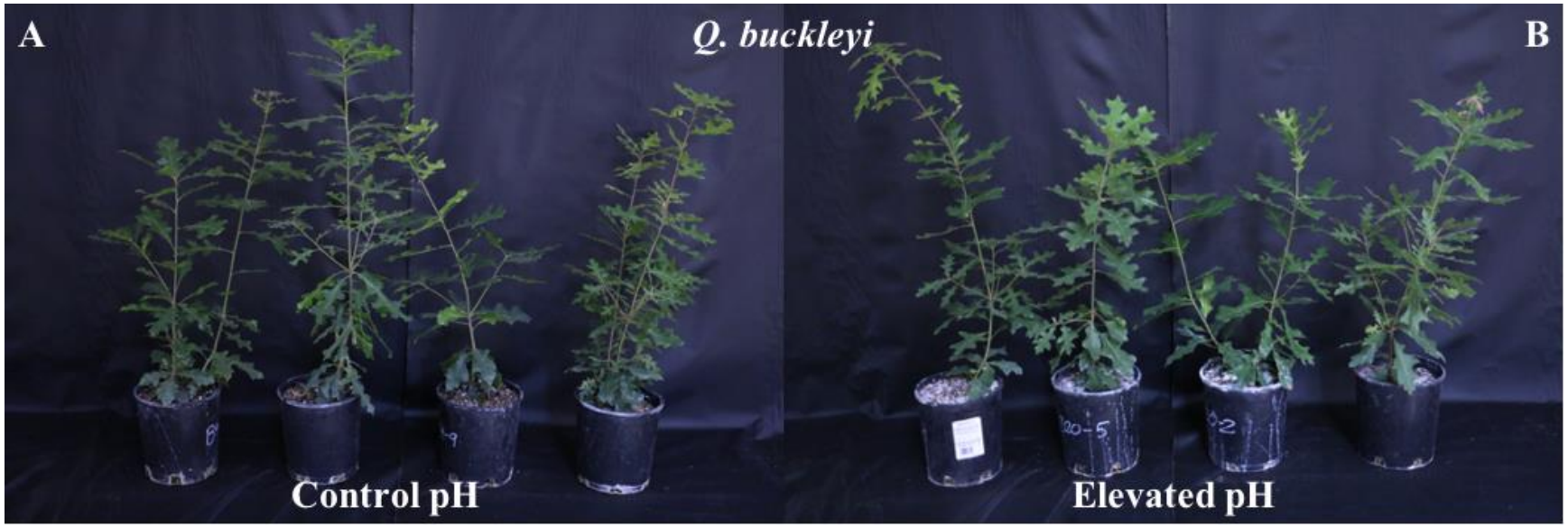

Figure 2.8 Expt. 1 2017: Four representative replications per treatment of *Q. buckleyi* taken after 172 d. (A) in the control pH substrate (mean pH = 6.13). (B) in the elevated pH substrate (mean pH = 7.40). Substrates began with either 0 kg·m^{-3} (control) or 11.9 kg·m^{-3} dolomitic lime (elevated) and received twice-weekly 120 mL drenches of either 0% (control) or 4.77% flowable $CaCO_3$ (elevated) over the course of the experiment.

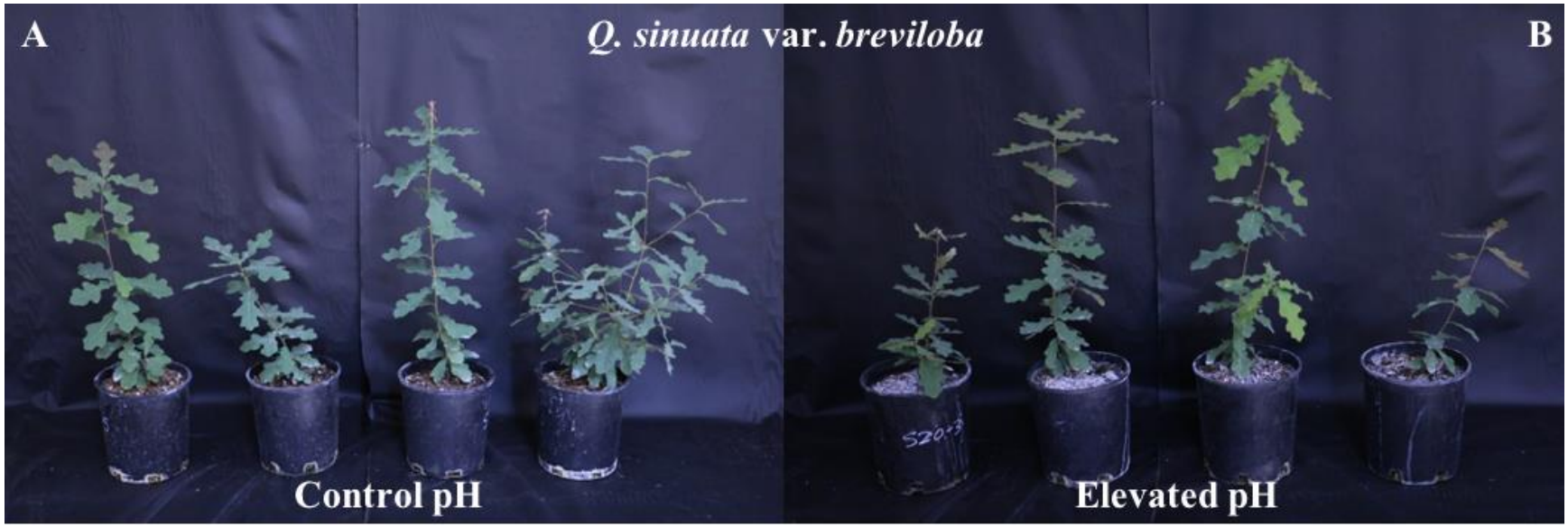

Figure 2.9 Expt. 1 2017: Four representative replications per treatment of *Q. sinuata var. breviloba* taken after 172 d (A) in the control pH substrate (mean pH = 6.13). (B) in the elevated pH substrate (mean pH = 7.40). Substrates began with either 0 $kg \cdot m^{-3}$ (control) or 11.9 $kg \cdot m^{-3}$ dolomitic lime (elevated) and received twice-weekly 120 mL drenches of either 0% (control) or 4.77% flowable $CaCO_3$ (elevated) over the course of the experiment.

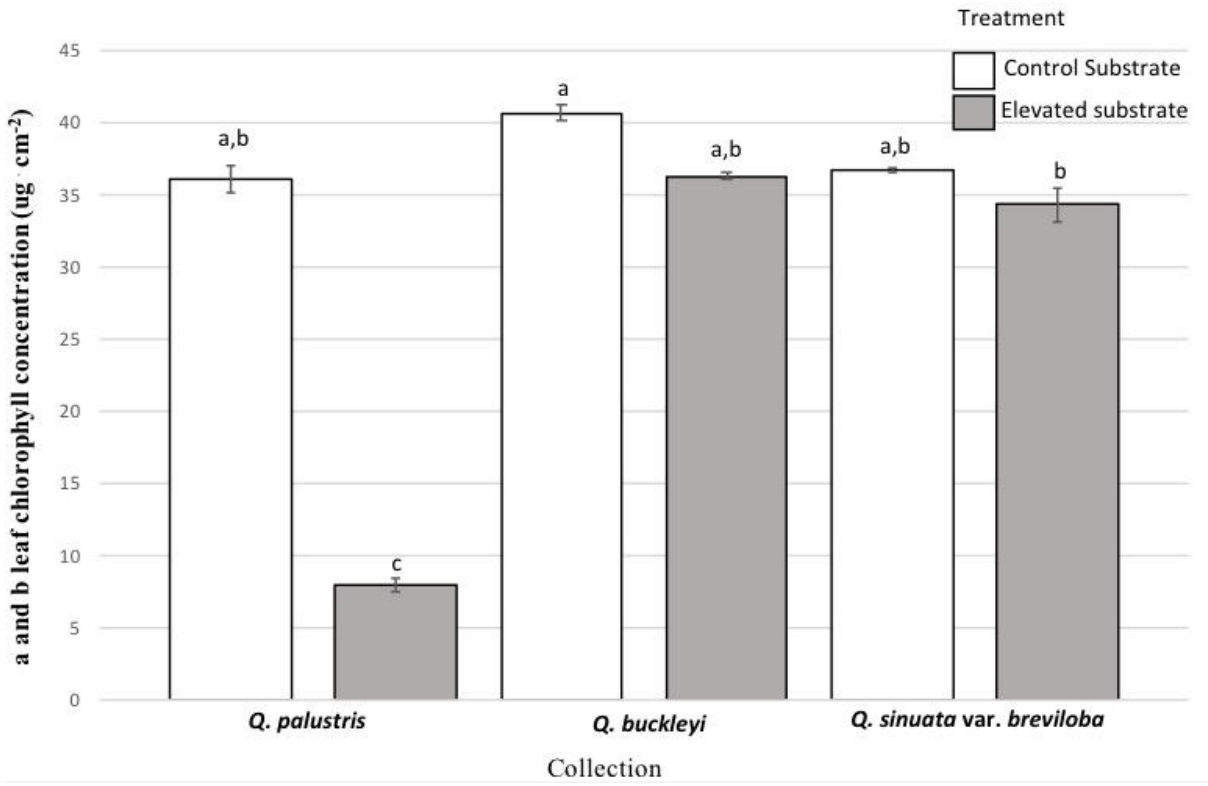

Figure 2.10 Expt. 1 2017: leaf chlorophyll a and b concentrations from leaves of *Q. palustris*, *Q. buckleyi*, and *Q. sinuata* var. *breviloba*. Substrates treatments began with either 0 kg·m^{-3} (control) or 11.9 kg·m^{-3} dolomitic lime (elevated) and received twice-weekly 120 mL drenches of either 0% (control) or 4.77% flowable $CaCO_3$ (elevated). Each bar represents in mean ± SE with n = 5 combined reps; expect for *Q. sinuata* var. *breviloba*, elevated = 3 combined reps. Data subjected to analysis of variance using LSM differences were assessed at $P \leq 0.05$ using Proc GLM/lsmeans option with Fisher' s Protected LSD in SAS® University Edition (2018).

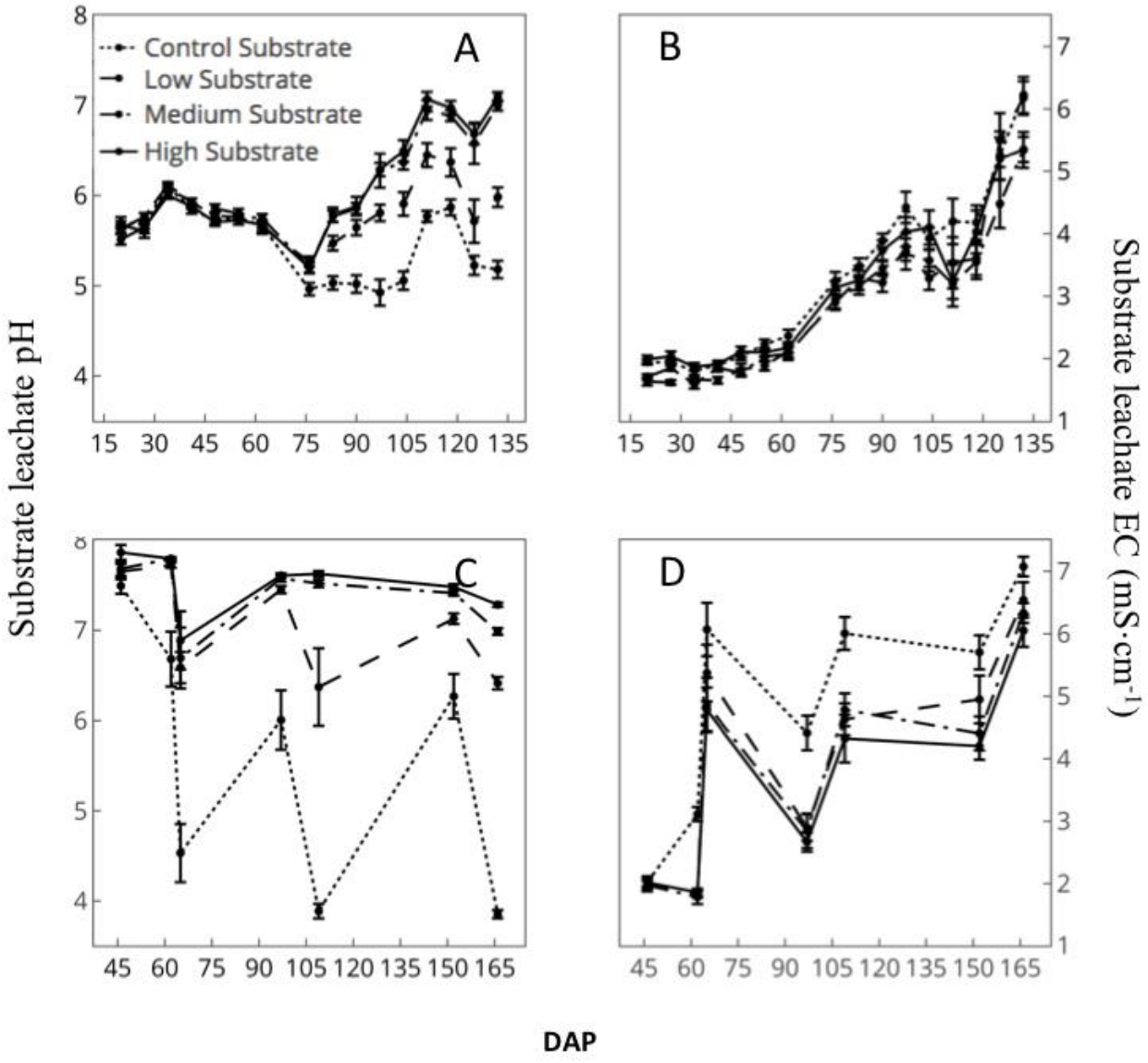

Figure 2.11 Expt. 2: Substrate leachate (A) pH and (B) electrical conductivity (EC) trends in 2016. (C) pH (control = 4.91, low = 6.79, medium = 7.24, and high = 7.38 the last 102 d in 2017) and (D) electrical conductivity (EC) trends for substrate treatments in 2017. Substrate treatments received twice-weekly 360mL drenches of 0% (control), 0.2% (low), 1.97% (medium), or 4.77% flowable $CaCO_3$ (high

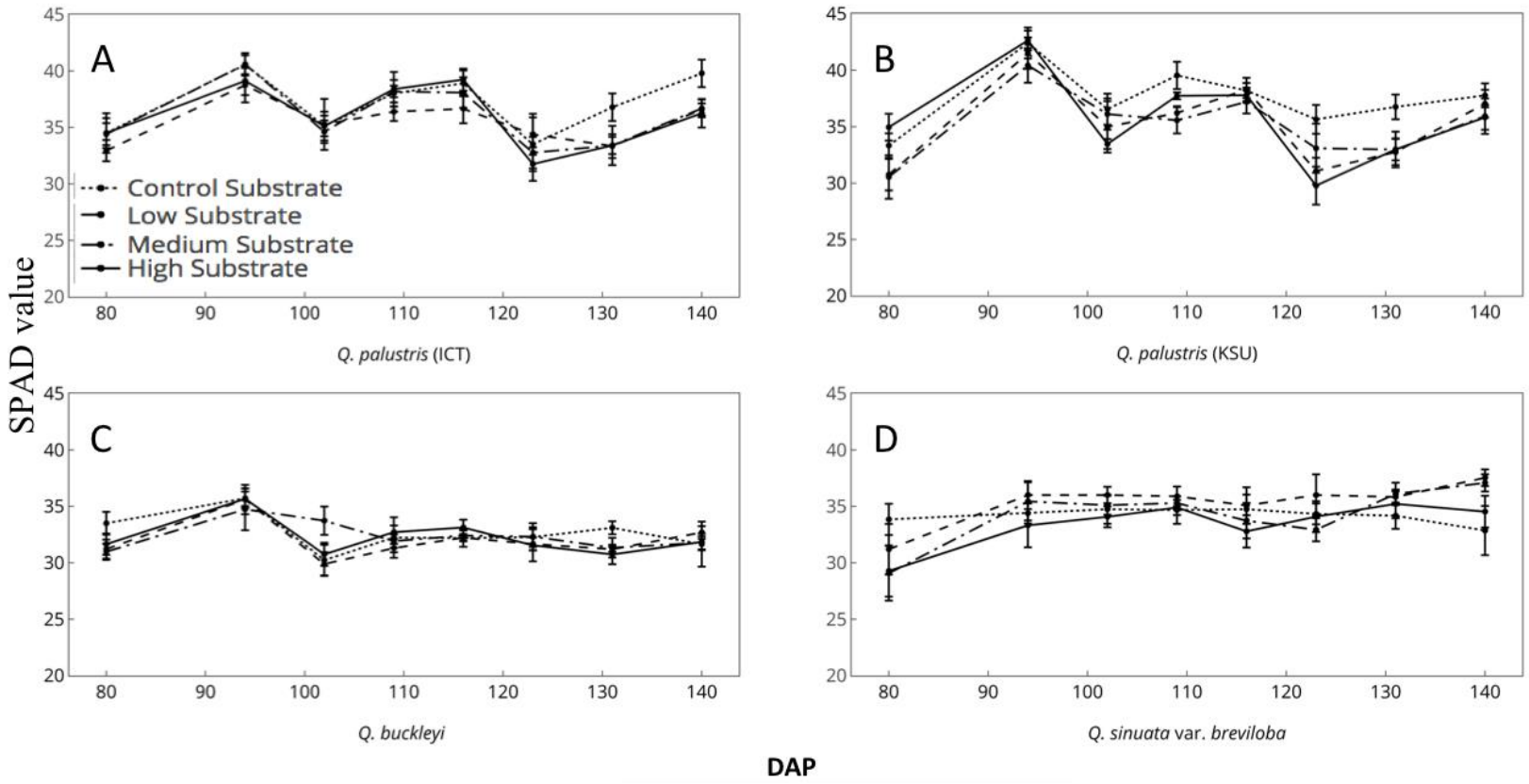

Figure 2.12 Expt. 2: Leaf SPAD trends for (A and B) trends from *Q. palustris* (ICT) and (KSU), respectively; (C and D) *Q. buckleyi*, and *Q. sinuata* var. *breviloba*, respectively for control, low, medium, and elevated substrate treatments over 2016. Control, low, medium, and high substrate treatments received twice-weekly 360 mL drenches of 0%, 0.2%, 1.97%, or 4.77% flowable CaCO3, respectively Each point represents in mean ± SE (n = 8), expect (D) (n=6) for control and low treatment.

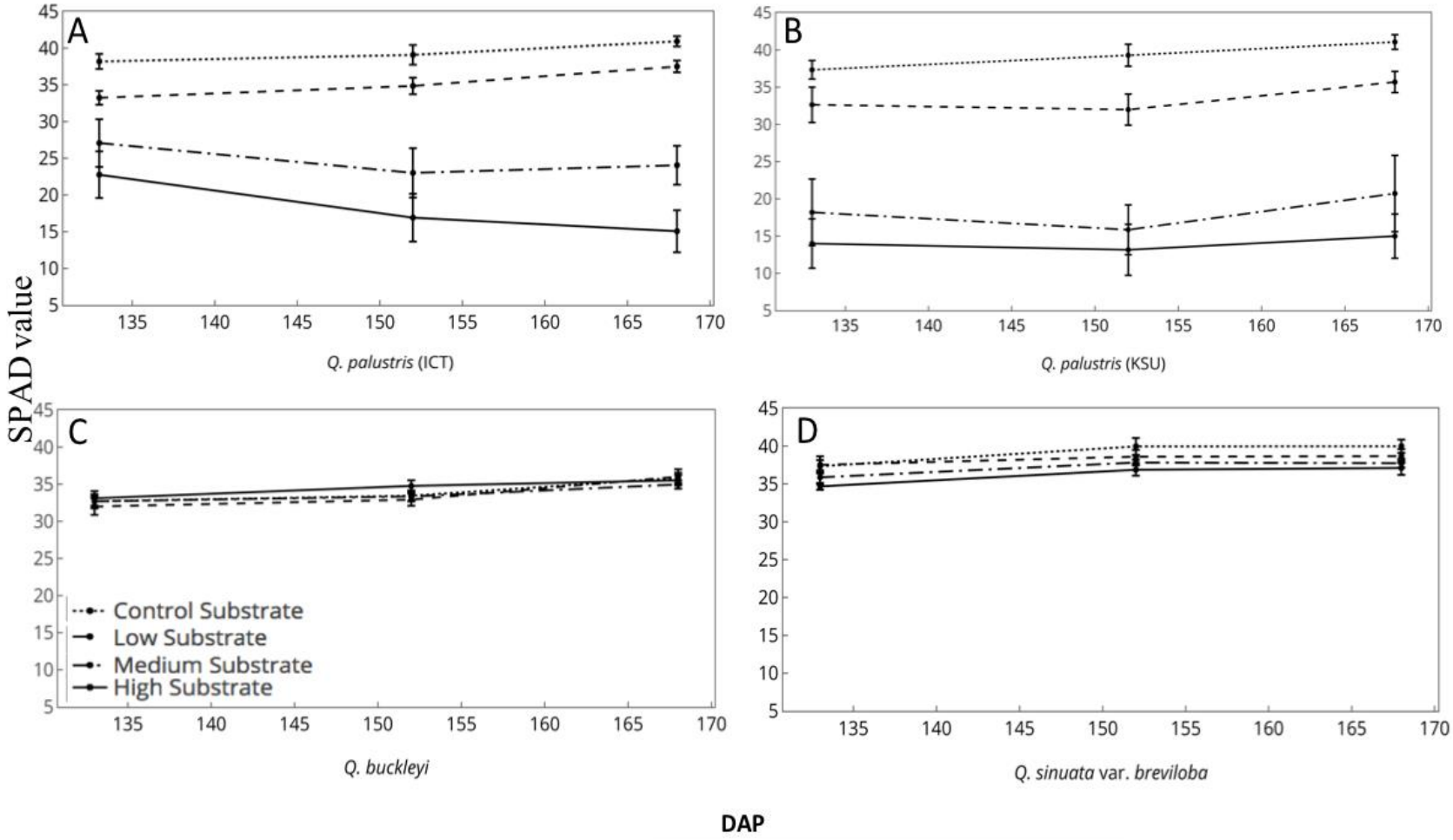

Figure 2.13 Expt. 2: Leaf SPAD trends for (A and B) trends from *Q. palustris* (ICT) and (KSU), respectively. (C and D) *Q. buckleyi*, and *Q. sinuata* var. *breviloba*, respectively in control (4.91), low (6.79), medium (7.24), and high (pH = 7.38) substrate pH treatments in 2017. Control, low, medium, and high substrate treatments received twice-weekly 360 mL drenches of either of 0%, 0.2%, 1.97%, or 4.77% flowable $CaCO_3$, respectively. Each point represents in mean ± SE (n = 8), expect (D) (n=6) for control and low treatments.

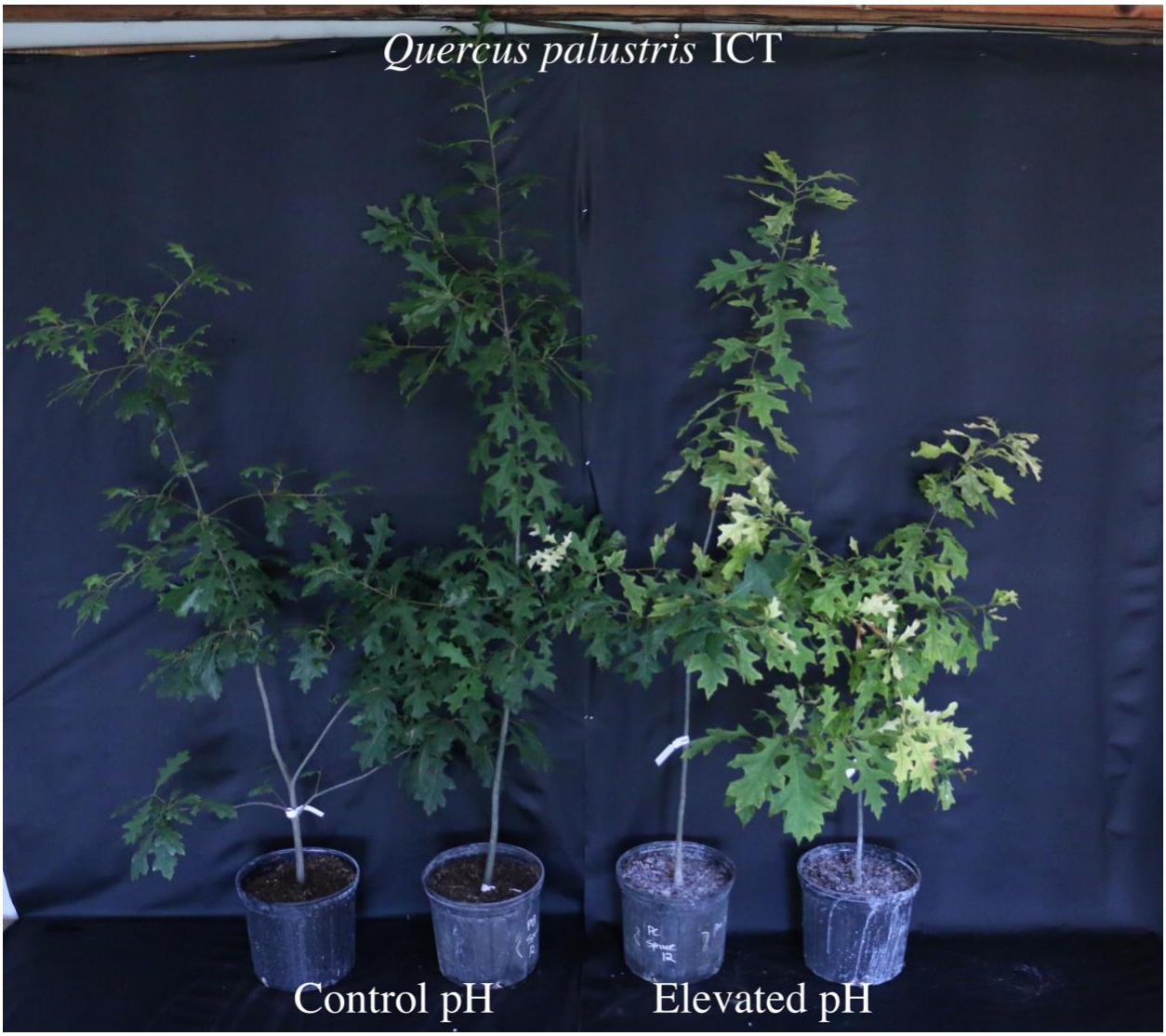

Figure 2.14 Expt. 2 2 Two representative replications of *Q. palustris* ICT per control (mean pH = 4.91) and high (pH = 7.38) pH substrate treatments taken after 169 d of. Control and high substrate treatments received twice-weekly 360 mL drenches of either 0% or 4.77% flowable $CaCO_3$, respectively. Notice stunted growth and IFC in the elevated treatment. Low and medium pH substrate treatments for *Q. palustris* ICT are not shown, and control, low medium and high pH substrate treatments for *Q. palustris* KSU are not shown.

Figure 2.15 Expt. 2 Two representative replications of *Q. buckleyi* for 2 replications per control (pH = 4.91) and high (pH = 7.38) pH substrate treatments taken after 169 d. Control and high substrate treatments received twice-weekly 360 mL drenches of either 0% or 4.77% flowable $CaCO_3$, respectively. Low and medium pH substrate treatments for *Q. buckleyi* are not pictured.

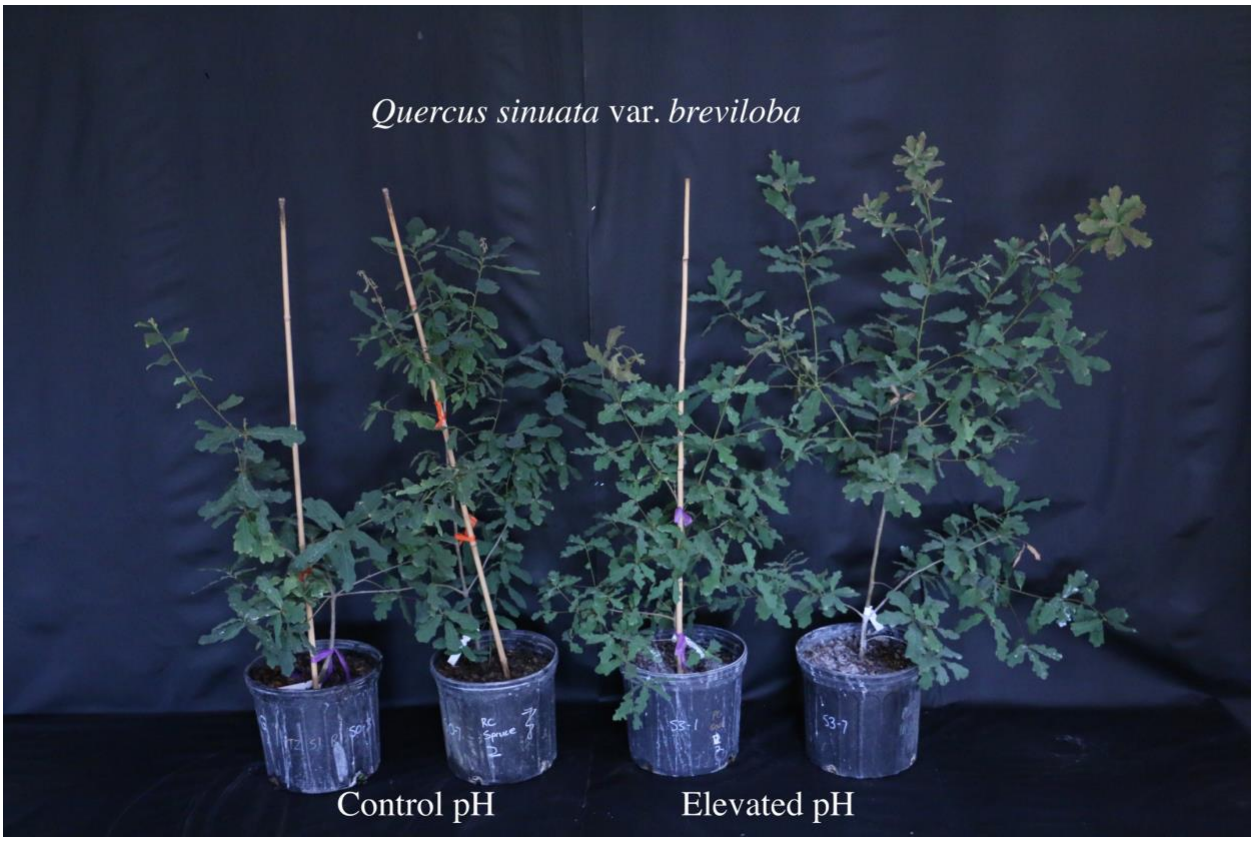

Figure 2.16 Expt. 2 Two representative replications of *Q. sinuata* var. *breviloba* for 2 replications per control (pH = 4.91) and high (pH = 7.38) pH substrate treatments taken after 169 d. Control and high substrate treatments received twice-weekly 360 mL drenches of either 0% or 4.77% flowable $CaCO_3$, respectively. Low and medium pH substrate treatments for *Q. sinuata* var. *breviloba* are not shown.

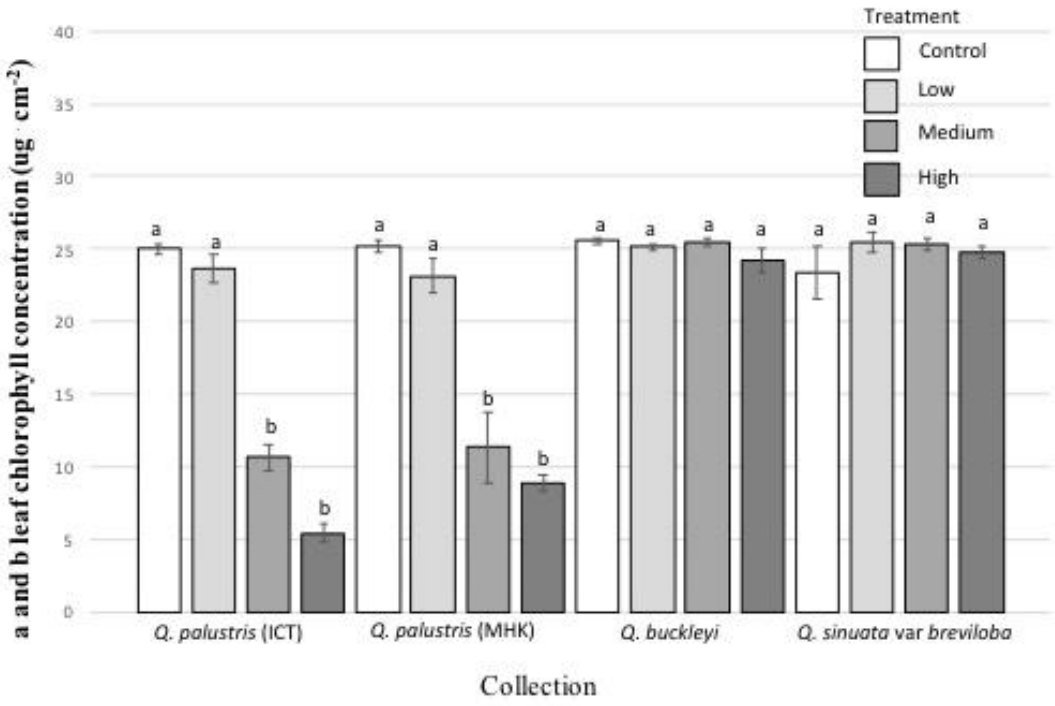

Figure 2.17 Expt. 2: leaf chlorophyll a and b concentrations after 171 d for *Q. palustris* (ICT and KSU), *Q. buckleyi,* and *Q. sinuata* var. *breviloba,* receiving twice-weekly 360 mL drenches of either 0%, 0.2%, 1.97%, or 4.77% flowable $CaCO_3$, Mean pH values the last 102 d: control (4.91), low (6.79), medium (7.24), and high (pH = 7.38) substrate pH treatments, respectively Each bar represents in mean ± SE (n = 4 combined replications) Data were subjected to analysis of variance using LSM differences were assessed at $P \leq 0.05$ using Proc GLM/lsmeans option with Fisher' s Protected LSD in SAS® University Edition (2018).

Table 2.1 Irrigation water analysis of John C. Pair Horticulture Center conducted by Brookside Laboratories, Inc., New Bremen, OH on 3 Aug. 2017.

Irrigation Water Analysis	
pH	7.45
Hardness (ppm)	396.63
Conductivity ($mS \cdot cm^{-1}$)	1.06
Total dissolved solids (ppm)	677.76
Calcium (ppm)	130.2
Iron (ppm)	1.16
Total alkalinity (ppm)	270.13
Bicarbonate (ppm)	329.61

Table 2.2 Expt. 1: Expt. 1: *P* values for effects of substrate drench treatment [(120 mL of 0, control or 4.77% $CaCO_3$, elevated)] on growth characteristics, leaf chlorophylls (a and b), micro- and macronutrients of leaves after 135 d (2016) and 175 d (2017) within a collection of *Q. palustris*, *Q. buckleyi*, and *Q. sinuata* var. *breviloba*.

Collection	Plant height		Stem diameter		Number of leaves		Total leaf area		Total leaf dry weight		Total shoot dry weight	
	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017
Q. palustris (ICT)	0.0465		0.0041 [z]		0.7218		0.0066 [z]		<0.0001 [z]		0.0002 [z]	
Q. palustris (KSU)	<0.0001	0.0041	<0.0001	0.0003	<0.0001	0.0334	<0.0001	0.0002	<0.0001	<0.0001	<0.0001	0.0027
Q. buckleyi	0.4801	0.0379	0.7966	0.3330	0.0077	0.9846	0.2527	0.3410	0.047	0.4478	0.2630	0.2799 [z]
Q. sinuata var. *breviloba*	0.796	0.4887	0.4685	0.0141	0.3148	0.1278	0.6556 [z]	0.2293	0.0125 [z]	0.1571	0.0299	0.0968

	SPAD (131 DAP)	(168 DAP)	leaf chlorophyll a and b		Fe		Mn		Cu		Zn	
	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017
Q. palustris (ICT)	<0.0001		0.0001		0.0204		0.0607		0.0001		0.0128	
Q. palustris (KSU)	<0.0001	<0.0001 [z]	<0.0001	0.0079	0.0031	0.0018	0.0252	<0.0001	0.0006	0.0053	0.0008	0.2537
Q. buckleyi	0.9941	0.4643	0.1259	0.2630	0.0341	0.4483	0.0041	0.0079 [z]	0.0002	<0.0001	0.001	0.0002
Q. sinuata var. *breviloba*	0.0535 [z]	0.0380	0.7353	0.1847	0.0159 [z]	0.0343	0.0051	0.0532	0.1508 [z]	0.0026	0.0006	0.0571 [z]

	N		C		P		K		Ca		Mg	
	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017
Q. palustris (ICT)	0.3901		0.0003		0.0043		<0.0001		0.0079 [z]		0.0003	
Q. palustris (KSU)	0.2798	0.6334	0.3105	0.0008	0.0022	0.0008	0.0002	<0.0001	<0.0001	0.2061	0.0075	0.0267
Q. buckleyi	0.0010	0.4606	0.0254	0.0326	0.0079 [z]	0.4683	0.8479	0.1746 [z]	0.0035	0.0582	0.1541	0.0009
Q. sinuata var. *breviloba*	0.2304	0.1108	0.3215	0.104	0.0005	0.2987	0.7222 [z]	0.4000 [z]	0.6011	0.6286	0.1496	0.0571 [z]

[z]If data were not normal, *P* values were assessed at $P \leq 0.05$ using Proc WMW Protected LSD.

If data were normal, *P* values were assessed at $P \leq 0.05$ using Proc ttest using SAS® University Edition 2018.

Table 2.3 Expt. 1: Effects of substrate drench treatment [(120 mL of 0%, control or 4.77% $CaCO_3$, elevated)] on growth characteristics after 135 d (2016) and 175 d (2017) within a collection of *Q. palustris*, *Q. buckleyi*, and *Q. sinuata* var. *breviloba*.

		Plant height (cm)		Plant stem diameter (mm)		Number of Leaves (count)		Total Leaf Area (cm^2)		Total leaf dry weight (g)		Total shoot dry weight (g)	
Collection	**Treatment** [z]	**2016**	**2017**	**2016**	**2017**	**2016**	**2017**	**2016**	**2017**	**2016**	**2017**	**2016**	**2017**
Q. palustris (ICT)	Control	66.0 ± 3.5 a [y]		12.5 ± 0.97 a		54.6 ± 7.1 a		2528.0 ± 222.2 a		18.9 ± 1.1 a		18.1 ± 0.96 a	
	Elevated	56.6 ± 2.7 b		9.98 ± 0.53 b		58.1 ± 6.4 a		1463.6 ± 206.4 b		8.31 ± 0.96 b		9.34 ± 1.2 b	
Q. palustris (KSU)	Control	70.8 ± 3.7a	44.2 ± 3.2 a	13.1 ± 0.34 a	9.42 ± 0.46 a	75.3 ± 5.6 a	32.9 ± 5.1 a	2728.3 ± 180.9 a	979.25 ± 87.8 a	17.8 ± 0.94 a	6.51 ± 0.64 a	16.5 ± 1.4 a	5.61 ± 0.77 a
	Elevated	40.6 ± 3.2 b	31.3 ± 2.2 b	7.13 ± 0.46 b	6.78 ± 0.38 b	24.2 ± 3.2 b	19.7 ± 1.9 b	599.20 ± 89.26 b	493.10 ± 56.8 b	3.99 ± 0.46 b	2.81 ± 0.36 b	3.54 ± 0.59 b	2.50 ± 0.30 b
Q. buckleyi	Control	72.7 ± 6.8 a	65.4 ± 4.6 a	6.96 ± 0.48 a	7.12 ± 0.33 a	102 ± 14 a	66.6 ± 7.7 a	1334.9 ± 148.8 a	1017.6 ± 70.2 a	10.5 ± 1.0 a	7.29 ± 0.56 a	6.91 ± 1.1 a	5.49 ± 0.38 a
	Elevated	65.8 ± 6.7 a	51.0 ± 4.5 b	7.12 ± 0.43 a	6.72 ± 0.23 a	53.7 ± 6.0 b	66.8 ± 6.7 a	1116.2 ± 113.8 a	924.66 ± 64.0 a	7.84 ± 0.70 b	6.73 ± 0.45 a	5.39 ± 0.77 a	4.27 ± 0.55 a
Q. sinuata var.	Control	45.7 ± 3.4 a	38.0 ± 8.4 a	4.19 ± 0.35 a	5.29 ± 0.47 a	56.5 ± 6.7 a	47.8 ± 11 a	633.30 ± 58.70 a	596.41 ± 162 a	4.33 ± 0.51 a	4.31 ± 1.2 a	1.70 ± 0.23 a	1.95 ± 0.54 a
breviloba	Elevated	47.6 ± 6.8a	31.0 ± 5.0 a	4.69 ± 0.61 a	3.47 ± 0.43 b	45.9 ± 7.8 a	31.4 ± 4.2 a	631.00 ± 111.7 a	367.27 ± 80.0 a	6.33 ± 0.67 b	2.36 ± 0.56 b	4.04 ± 0.88 b	0.861 ± 0.26 a

[z] The treatment column refers to substrate treatments and their mean leachate pH values for 2016 and 2017 [(control = 5.58 and 6.13, respectively) and (elevated = 6.72 and 7.40, respectively)].

[y] Treatment means ± 1 SE of the mean. Values in a column and within a collection sharing the same letter not differ statistically. *P* values were assessed at $P \leq 0.05$ using Proc ttest. If data were not normal, *P* values were assessed at $P \leq 0.05$ using Proc Wilcoxon-Mann-Whitney test using SAS® University Edition 2018. Refer to Table 2.4 for *P* value assessment.

Table 2.4 Expt. 1: Effects of substrate drench treatments [(120 mL of 0%, control or 4.77% $CaCO_3$, elevated)] on macronutrients from leaves after 135 d (2016) and 187 d (2017) within a collection of *Q. palustris*, *Q. buckleyi*, and *Q. sinuata* var. *breviloba*.

		N %		C %		P %		K %		Ca %		Mg %	
Collection	**Treatment** [z]	**2016**	**2017**	**2016**	**2017**	**2016**	**2017**	**2016**	**2017**	**2016**	**2017**	**2016**	**2017**
Q. palustris (ICT)	Control	2.59 ± 0.12 a [y]		45.87 ± 0.096 a		0.142 ± 0.0094 b		0.924 ± 0.034 b		0.591 ± 0.049 b		0.188 ± 0.012 b	
	Elevated	2.73 ± 0.075 a		45.06 ± 0.093 b		0.191 ± 0.0080 a		1.62 ± 0.090 a		0.827 ± 0.041 a		0.294 ± 0.012 a	
Q. palustris (KSU)	Control	2.51 ± 0.061 a	2.65 ± 0.14 a	45.56 ± 0.14 a	46.00 ± 0.41 a	0.139 ± 0.0044 b	0.119 ± 0.011 b	0.830 ± 0.032 b	1.03 ± 0.053 b	0.633 ± 0.030 b	0.612 ± 0.072 a	0.183 ± 0.0067 b	0.205 ± 0.022 b
	Elevated	2.40 ± 0.063 a	2.56 ± 0.12 a	45.74 ± 0.091 a	44.82 ± 0.18 b	0.175 ± 0.0069 a	0.181 ± 0.0050 a	1.352 ± 0.077 a	2.03 ± 0.075 a	0.934 ± 0.021 a	0.720 ± 0.033 a	0.243 ± 0.016 a	0.267 ± 0.0084 a
Q. buckleyi	Control	2.32 ± 0.068 a	2.11 ± 0.068 a	46.10 ± 0.098 a	46.08 ± 0.11 a	0.246 ± 0.046 a	0.109 ± 0.0080 a	0.790 ± 0.060 a	0.860 ± 0.063 a	0.791 ± 0.042 b	0.685 ± 0.041 a	0.340 ± 0.018 a	0.313 ± 0.012 a
	Elevated	1.93 ± 0.036 b	2.04 ± 0.060 a	45.92 ± 0.14 b	45.78 ± 0.045 b	0.121 ± 0.0080 b	0.121 ± 0.012 a	0.804 ± 0.037 a	1.05 ± 0.080 a	1.18 ± 0.084 a	0.842 ± 0.058 a	0.299 ± 0.018 a	0.243 ± 0.0065 b
Q. sinuata var.	Control	2.06 ± 0.051 a	2.26 ± 0.041 a	45.11 ± 0.27 a	45.37 ± 0.31 a	0.273 ± 0.019 a	0.139 ± 0.0020 a	0.804 ± 0.047 a	0.760 ± 0.026 a	0.971 ± 0.065 a	0.848 ± 0.050 a	0.250 ± 0.020 a	0.263 ± 0.016 a
breviloba	Elevated	1.99 ± 0.012 a	1.99 ± 0.12 a	45.25 ± 0.049 a	45.16 ± 0.17 a	0.156 ± 0.0080 b	0.174 ± 0.028 a	0.760 ± 0.03 a	0.890 ± 0.10 a	0.928 ± 0.045 a	0.967 ± 0.10 a	0.213 ± 0.012 a	0.211 ± 0.0077 a

[z] The treatment column refers to substrate treatments and their mean leachate pH values for 2016 and 2017 [(control = 5.58 and 6.13, respectively) and (elevated = 6.72 and 7.40, respectively)].

[y] Treatment means ± 1 SE of the mean. Values in a column and within a collection sharing the same letter not differ statistically. *P* values were assessed at $P \leq 0.05$ using Proc ttest. If data were not normal, *P* values were assessed at $P \leq 0.05$ using Proc Wilcoxon-Mann-Whitney test using SAS® University Edition 2018. Refer to Table 2.4 for *P* value assessment.

Table 2.5 Expt. 1: Effects of substrate drench treatment [(120 mL of 0%, control or 4.77% $CaCO_3$, elevated)] on micronutrient concentrations from leaves after 135 d (2016) and 175 d (2017) within a collection of *Q. palustris*, *Q. buckleyi* and *Q. sinuata* var. *breviloba*.

		Fe (mg·kg^{-1})		Mn (mg·kg^{-1})		Cu (mg·kg^{-1})		Zn (mg·kg^{-1})	
Collection	**Treatment** [z]	**2016**	**2017**	**2016**	**2017**	**2016**	**2017**	**2016**	**2017**
Q. palustris (ICT)	Control	63.8 ± 5.7 a [y]		1040 ± 163 a		4.32 ± 0.37 a		49.3 ± 3.7 b	
	Elevated	43.5 ± 2.0 b		657.0 ± 64 a		1.60 ± 0.13 b		62.0 ± 1.5 a	
Q. palustris (KSU)	Control	64.9 ± 4.5 a	35.3 ± 4.8 a	1170 ± 139 b	2070 ± 19 a	5.36 ± 0.45 a	3.04 ± 0.37 a	54.7 ± 2.0 b	53.9 ± 6 a
	Elevated	44.5 ± 1.8 b	12.0 ± 1.8 b	2080 ± 301 a	267 ± 76 b	2.36 ± 0.33 b	1.56 ± 0.12 b	81.7 ± 4.8 a	45.6 ± 3.2 a
Q. buckleyi	Control	208 ± 37.1 a	44.8 ± 3.5 a	1376 ± 201 a	588 ± 66 a	7.22 ± 0.52 a	2.80 ± 0.071 a	77.1 ± 6.8 a	33.0 ± 1.8 a
	Elevated	92.4 ± 6.3 b	41.7 ± 1.5 a	518.0 ± 78 b	73.2 ± 36 b	3.16 ± 0.32 b	1.36 ± 0.19 b	25.1 ± 1.9 b	20.4 ± 1.0 b
Q. sinuata var. *breviloba*	Control	134 ± 25.5 a	39.7 ± 4.5 a	1518 ± 227 a	573 ± 125	4.72 ± 0.41 a	3.13 ± 0.28 a	78.6 ± 6.9 a	53.0 ± 3.1 a
	Elevated	86.7 ± 4.2 b	27.4 ± 1.7 b	554.0 ± 109 b	72.3 ± 21	3.92 ± 1.4 b	1.43 ± 0.17 b	37.6 ± 3.0 b	31 ± 2.9 b

[z] The treatment column refers to substrate treatments and their mean leachate pH values for 2016 and 2017 [(control = 5.58 and 6.13, respectively) and (elevated = 6.72 and 7.40, respectively)].

[y] Treatment means ± 1 SE of the mean. Values in a column and within a collection sharing the same letter not differ statistically. *P* values were assessed at $P \leq 0.05$ using Proc ttest. If data were not normal, *P* values were assessed at $P \leq 0.05$ using Proc Wilcoxon-Mann-Whitney test using SAS® University Edition 2018. Refer to Table 2.4 for *P* value assessment.

Table 2.6 Expt. 2: *P* values for effects of substrate treatments [z] on growth characteristics, leaf SPAD, leaf chlorohylls (a and b), and micro- and macronutrients for collections of *Q. palustris, Q. buckleyi, and Q. sinuata var. breviloba*.

Collection	Plant height	Stem diameter	Number of leaves	Total leaf area	Leaf dry weight	Shoot dry weight
Q. palustris (ICT)	0.0515 [y]	0.0965	0.4270	0.0036	0.0001	0.0002
Q. palustris (KSU)	0.4002	<0.0001	0.3122	0.0932	0.0105	0.0046
Q. buckleyi	0.5117	0.1957	0.7933	0.1367	0.4437	0.5855
Q. sinuata var. *breviloba*	0.6591	0.4084	0.8773	0.9182	0.9422	0.9573

	SPAD (131 DAP)	leaf chlorophyll a and b	Fe	Mn	Cu	Zn
Q. palustris (ICT)	<0.0001	<0.0001	0.1127	<0.0001	<0.0001	0.2577
Q. palustris (KSU)	<0.0001	<0.0001	0.0004	<0.0001	0.0044	0.5417
Q. buckleyi	0.7770	0.4969	0.6576	<0.0001	0.0171	0.0060
Q. sinuata var. *breviloba*	0.1264	0.4385	0.7415	<0.0001	0.0015	0.0020

	N	C	P	K	Ca	Mg
Q. palustris (ICT)	0.4117	<0.0001	0.0221	<0.0001	0.0092	0.1794
Q. palustris (KSU)	0.4281	<0.0001	0.0060	<0.0001	0.0143	0.2564
Q. buckleyi	0.3725	0.0352	0.0032	0.0665	0.0292	0.7089
Q. sinuata var. *breviloba*	0.1205	0.5092	0.5092	0.9754	0.0881	0.7846

[z] Treatments consisted of a 360 mL drench of; 0% (control), 0.2% (low), 1.97% (medium), and 4.77% (high) $CaCO_3$.

[y] *P* values were assessed at $P \leq 0.05$ using Proc GLM ls/means with Fishers Protected LSD using SAS® University Edition 2018.

Table 2.7 Expt. 2: Effects of substrate treatment [z] on growth characteristics for collections of *Q. palustris, Q. buckleyi, and Q. sinuata var. breviloba*.

Collection	Treatment [y]	Plant height (cm)	Plant stem diameter (mm)	Number of Leaves (count)	Total Leaf Area (cm^2)	Leaf dry weight (g)	Shoot dry weight (g)
Q. palustris (ICT)	Control	164.3 ± 7.29 a [x]	19.85 ± 0.630 a	232.1 ± 22.0 a	8455.2 ± 339 a	56.40 ± 3.24 a	110 ± 6.11 ab
	Low	174.4 ± 9.78 a	22.37 ± 0.973 a	222.5 ± 20.1 a	7865.7 ± 632 a	48.61 ± 3.44 ab	115 ± 7.39 a
	Medium	156.0 ± 5.95 a	22.51 ± 0.606 a	202.4 ± 24.1 a	6890.1 ± 587 ab	40.08 ± 3.88 bc	92.8 ± 6.04 b
	High	143.4 ± 7.76 a	21.68 ± 0.804 a	185.6 ± 17.7 a	5530.8 ± 468 b	31.33 ± 2.76 c	71.9 ± 4.9 c
Q. palustris (KSU)	Control	160.9 ± 8.80 a	25.7 ± 0.500 a	214.8 ± 31.8 a	7131.8 ± 540 a	44.83 ± 3.37 a	106 ± 9.80 a
	Low	160.4 ± 12.4 a	24.43 ± 0.517 a	196.1 ± 12.9 a	7024.8 ± 519 a	42.63 ± 3.08 ab	93.0 ± 5.95 ab
	Medium	147.4 ± 11.5 a	19.24 ± 0.846 b	187.6 ± 16.1 a	6120.9 ± 587 a	33.81 ± 3.18 bc	75.8 ± 8.33 bc
	High	140.1 ± 6.69 a	20.48 ± 0.721 b	159.9 ± 15.1 a	5469.0 ± 433 a	31.36 ± 2.70 c	66.6 ± 5.10 c
Q. buckleyi	Control	157.0 ± 12.8 a	18.83 ± 0.880 a	398.6 ± 63.3 a	5401.1 ± 754 a	41.61 ± 5.93 a	69.9 ± 12.4 a
	Low	158.7 ± 9.62 a	20.22 ± 0.745 a	421.6 ± 58.3 a	6918.8 ± 471 a	50.06 ± 3.62 a	82.2 ± 5.64 a
	Medium	137.4 ± 11.6 a	20.29 ± 0.985 a	335.6 ± 33.0 a	5301.0 ± 517 a	41.66 ± 3.97 a	70.7 ± 9.56 a
	High	147.7 ± 10.1 a	17.93 ± 0.908 a	397.3 ± 75.2 a	5119.7 ± 445 a	42.38 ± 3.01 a	64.5 ± 7.74 a
Q. sinuata var. *breviloba*	Control	75.90 ± 14.6 a	9.740 ± 1.93 a	204.2 ± 79.8 a	2212.9 ± 777 a	15.81 ± 5.79 a	17.4 ± 7.48 a
	Low	73.60 ± 12.0 a	10.37 ± 1.29 a	159.6 ± 31.2 a	2108.7 ± 464 a	14.89 ± 3.32 a	16.7 ± 5.56 a
	Medium	96.10 ± 8.93 a	12.01 ± 1.29 a	209.0 ± 41.3 a	2651.3 ± 380 a	18.40 ± 2.81 a	20.6 ± 4.17 a
	High	80.40 ± 17.4 a	8.960 ± 1.24 a	232.4 ± 76.2 a	2327.1 ± 664 a	17.46 ± 4.87 a	17.8 ± 5.59 a

[z] Treatments consisted of a 360 mL drench of; 0% (control), 0.2% (low), 1.97% (medium), and 4.77% (high) $CaCO_3$.

[y] The treatment column refers to substrate treatments and their mean leachate pH values (control = 4.91, low = 6.79, medium = 7.24, and high =7.38) last 102 d.

[x] Treatment means ± 1 SE of the mean. Values in the same column within a collection sharing the same letter not differ statistically. *P* values and LSM differences were assessed at $P \leq 0.05$ using Proc GLM/lsmeans option with Fisher's Protected LSD in SAS® University Edition (2018). Refer to Table 2.12 for *P* value assessment.

Table 2.9 Expt. 2: Effects of substrate treatments [z] on growth characteristics, leaf SPAD, leaf chlorophylls (a and b), and micronutrients of leaves for collections of *Q. palustris, Q. buckleyi and Q. sinuata var. breviloba*.

Collection	Treatment [y]	Fe (mg·kg^{-1})	Mn (mg·kg^{-1})	Cu (mg·kg^{-1})	Zn (mg·kg^{-1})
Q. palustris (ICT)	Control	43.8 ± 2.1 a [x]	982 ± 160 a	2.90 ± 0.25 a	56.4 ± 1.8 a
	Low	45.9 ± 1.8 a	859 ± 110 a	1.33 ± 0.16 b	65.9 ± 3.8 a
	Medium	28.3 ± 4.4 a	160 ± 30 b	1.10 ± 0.15 b	56.4 ± 2.2 a
	High	45.5 ± 8.8 a	131 ± 13 b	1.35 ± 0.14 b	64.1 ± 5.9 a
Q. palustris (KSU)	Control	58.5 ± 4.7a	1064 ± 91 a	3.30 ± 0.41 a	59.9 ± 3.4 a
	Low	43.3 ± 5.9 b	644 ± 33 b	1.55 ± 0.050 b	60.4 ± 1.2 a
	Medium	29.7 ± 5.2 bc	216 ± 27 c	1.50 ± 0.22 b	56.3 ± 3.5 a
	High	19.2 ± 2.1 c	148 ± 11 c	1.30 ± 0.32 b	61.8 ± 2.1 a
Q. buckleyi	Control	58.0 ± 7.1 a	661 ± 83 a	3.30 ± 0.53 a	40.8 ± 4.6 a
	Low	61.4 ± 9.9 a	453 ± 87 b	2.15 ± 0.30 b	34.1 ± 3.5 ab
	Medium	49.7 ± 3.4 a	123 ± 23 c	1.67 ± 0.13 b	26.7 ± 2.3 bc
	High	54.5 ± 4.8 a	77.2 ± 12 c	1.47 ± 0.22 b	21.1 ± 1.9 c
Q. sinuata var.	Control	43.0 ± 7.3 a	895 ± 130 a	3.80 ± 0.70 a	38.6 ± 1.6 a
breviloba	Low	44.4 ± 0.70 a	660 ± 60 b	3.75 ± 0.35 a	45.0 ± 6.4 a
	Medium	47.8 ± 3.8 a	84.2 ± 16 c	1.68 ± 0.14 b	26.0 ± 1.7 b
	High	42.1 ± 3.7 a	102 ± 42 c	1.57 ± 0.18 b	26.0 ± 1.4 b

[z] Treatments consisted of a 360 mL drench of; 0% (control), 0.2% (low), 1.97% (medium), and 4.77% (high) $CaCO_3$.

[y] The treatment column refers to substrate treatments and their mean leachate pH values (control = 4.91, low = 6.79, medium = 7.24, and high =7.38) last 102 d.

[x] Treatment means ± 1 SE of the mean. Values in the same column within a collection sharing the same letter not differ statistically. *P* values and LSM differences were assessed at $P \leq 0.05$ using Proc GLM/lsmeans option with Fisher's Protected LSD in SAS® University Edition (2018). Refer to Table 2.12 for *P* value assessment.

Chapter 3 - Effects of High pH Substrate on Chlorophyll Content, Foliar Chlorosis, and Plant Growth of River Birch from Iowa Grown Provenances

Abstract

River birch (*Betula nigra* L.) is a common landscape shade tree known to develop iron deficiency interveinal leaf chlorosis (IFC) when grown in high pH and calcium carbonate ($CaCO_3$) soils. While variation in symptomology has been observed, provenances endemic to high pH soils may not always display chlorosis. With increased interest for environmentally sustainable landscape selections, screenings of *B. nigra* sources could determine their potential adaptability to high pH and $CaCO_3$ soils. The first study (Expt. 1) evaluated open-pollinated (OP) seedlings of Iowa provenances and OP 'BNMTF' of *B. nigra* in an elevated pH substrate. A second study (Expt. 2) evaluated clones from selected Iowa provenances, 'BNMTF', 'Cully', and OP 'BNMTF' in an elevated pH substrate. Twice-weekly 120 mL drenches of 4.8% $CaCO_3$ were used to maintain an elevated substrate pH. In Expt. 1, leaf chlorophyll was reduced by 36% to 16.95 $\mu g \cdot cm^{-2}$ in elevated pH substrate (7.57) compared to 6.39 $\mu g \cdot cm^{-2}$ in the control pH substrate with differences in seed sources also observed. A seed source from Bearbower Sand Prairie, Buchanan Co., IA (BSP3) had the greatest leaf chlorophyll content (25.86 $\mu g \cdot cm^{-2}$), but was not statistically greater than two sources from Clemons Creek WMA, Washington Co., IA (23.90 $\mu g \cdot cm^{-2}$, CCWMA1 and 22.76 $\mu g \cdot cm^{-2}$, CCWMA2). Additionally, these three seed sources had greater leaf chlorophyll than OP 'BNMTF' (18.62 $\mu g \cdot cm^{-2}$). Total leaf iron (Fe) concentrations were dramatically reduced in the elevated pH substrate. In Expt. 2, leaf chlorophyll was reduced by 32% 19.40 $\mu g \cdot cm^{-2}$ in elevated substrate pH (7.00) compared

ton28.73 μg·cm^{-2} in the control (5.29) and differences between sources were observed. An Iowa clone, CCWMA3, in the elevated substrate pH was less chlorotic than the other some other sources (leaf chlorophyll = 26.78 μg·cm^{-2}), but had no greater leaf chlorophyll content than a trade standard (25.70 μg·cm^{-2}, 'BNMTF'), a source from Ciha Fen, Johnson Co., IA (24.95 μg·cm^{-2}, CF1), and a source from Princeton Wildlife Management Area (24.13 μg·cm^{-2}, PWMA1). 'Cully', a popular cultivar of *B. nigra*, had lower leaf chlorophyll (21.87 μg·cm^{-2}) than CCWMA2 and 'BNMTF', and displayed dramatic IFC symptoms. Similar to Expt. 1, total leaf Fe content was reduced in elevated substrate compared to their controls for all germplasm sources. Based on our studies, these Iowa provenances did not sequester more substrate leaf Fe than the industry standards, but two selections (BSP3, Expt. 1 and CCWMA3, Expt. 2) were perhaps more Fe efficient, because they were considerably less chlorotic than OP 'BNMTF' and 'Cully' in Expt. 2. These Iowa seed sources and clones should be further evaluated in field studies to determine their extent of Fe-use efficiency in high pH soils compared to popular industry cultivars.

Introduction

Birch (*Betula* L.) rank as the third most popular deciduous shade tree genera only behind maple (*Acer* L.) and oak (*Quercus* L.), and they contribute to roughly $25 million in annual sales which is a significant portion (4.5%) of all annual deciduous shade tree sales in United States (USDA Agriculture Census, 2014). The growth habit, desirable bark texture and color, leaf size and color have made river birch (*Betula nigra* L.) and its cultivars a popular choice as an ornamental shade tree throughout Northeastern and Midwestern landscapes in the United States. Cultivars of *B. nigra* have long been touted as fast-growing, flood tolerant, drought resistant, and insect and disease resistant alternatives that out-compete other species of *Betula* L. and their

cultivars grow in throughout U.S. Department of Agriculture (USDA) 2012 Hardiness Zones 5 to 9 landscapes (Cully, 1979; Dirr, 2011). Unfortunately with the increased use of these *B. nigra* in urban landscapes, Fe-deficiency interveinal foliar chlorosis (IFC) has been a prominent drawback. When planted in soils with a pH 6.5 and above, IFC can develop, because as soil pH increases, the availability of micronutrients, especially Fe, for root uptake diminishes (Lindsay 1984; Lindsay and Schawb, 1982; Whitman and Ranney, 1994 and 1995). Furthermore, construction related activities due to urbanization have worsened the problem, because now many of these soils having high pH are characterized by high $CaCO_3$ contents which further exacerbate IFC in pH sensitive woody ornamentals (Craul, 1992; Kimihiro and Kawahigashi, 2015; Pregitzer et al., 2016; Scalenghe and Marsan, 2009). When soil bicarbonate (HCO_3^-) concentration is high, substantial buffering is achieved, and any potential reduction in rhizosphere pH by root-rhizosphere acidification (Strategy I plants), for increased bioavailability of pH-dependent nutrients, diminishes (Brancadoro et al., 1995). Strategy I rhizosphere acidification has been observed in many woody species (Gogorcena et al., 2001; Romera et al., 2008; Venturas et al., 2014). It has not yet been directly confirmed in species of *Betula,* even though native stands of silver birch (*Betula pendula* Roth.) have been observed to decrease rhizosphere pH when the bulk pH of soils approach 6.9 to 7.0 (Rosenvald et al., 2011). In addition, Fe reduction at the root rhizosphere interface is another crucial step before Fe uptake (Marschner, 2011). High soil HCO_3^- and insufficient nutrient contents can impede optimal performance of root ferric chelate reductase (FCR) and lessen the plants ability to signal for Fe deficiency (Felle, 1998; Schmidt, 2006; Lucena, 2000; Lucena et al., 2007).

Experimentally, a range of interspecific susceptibility to IFC has been observed in *Betula*. Yellow birch (*Betula alleghaniensis* Britt.) has a greater tolerance to IFC induced at high pH,

while other North America species, such as sweet birch (*Betula lenta* L.), river birch (*Betula nigra* L.), and paper birch (*Betula papyrifera* Marshall), are generally more susceptible (McNamara and Pellet, 2001). The first patented cultivar of *B. nigra*, 'Cully' originated from cuttings of specimen in a St. Louis, MO landscape of unknown parentage. It has been a popular trade standard, because of its rapid growth, dense branching habit, large dark green leaves, and exfoliating light tan to creamy white which flakes in large sheets (Cully, 1978). Unfortunately, 'Cully' along with seedlings from various sources, may develop IFC in the landscape from the lack of Fe availability in alkaline soils (Carlson, 2003). Screening for provenances of *B. nigra* tolerant to high pH and IFC has not been formally conducted, but reports argue more recently derived cultivars, 'Dickinson' and 'Whit XXV', are tolerant to high pH (Herman, 2009; Herman and Quam, 2006; Whitcomb, 2006).

Cultivar development of *B. nigra* tolerant to high pH has been slow, because selection for uniform habit, ornamental bark and foliage features, and drought tolerance has taken precedence and two industry standards, 'BNMTF' and 'Cully' are readily available. Since asexual propagation techniques (tissue culture and softwood cuttings) are common nursery practices for *B. nigra* production, the possible variation in adaptive features for tolerance to high pH from distinctly separated provenances may not yet be fully observed (Barnes, 2002). Additionally, hopes for developing interspecific hybrids of *B. nigra* with tolerance to high pH is complex, because Fe deficiency tolerance is controlled by a complex set of quantitative genes, many transcription factors, and hormone signaling pathways (Peiffer et al., 2012; Rogers and Guerinot; 2002; Wild; 2016; Wiren and Bennett; 2016). Furthermore, *B. nigra* is difficult to successfully hybridize with other species of *Betula* (Clausen, 1966; Hoch et al., 2002; Santamour, 1982).

To facilitate the selection of tolerance to high pH and IFC resistant provenances of *B. nigra*, it is assumed that interspecific variation for soil pH adaptability exists, because the native distribution of *B. nigra* spans from Florida to as far west as Kansas in the U.S. (Kartsez, 2015; Koevenig, 1976). Since its distribution is expansive, intraspecific variation for tolerance to alkaline soils would likely be found in populations growing in warmer and drier regions on alkaline soils where ferric Fe (Fe^{+3}) is more abundant and where $CaCO_3$ is not leached from the soil profile (Breeman and Van Protz, 1988; Slessarev et al., 2016). Thus, screening provenances of *B. nigra* native to warmer and drier climates and calcareous soils could increase the chances of identifying intraspecific germplasm adaption to high soil pH and tolerance to IFC (McNamara and Pellet, 2001; Slessarev et al., 2016).

The objective of this work was to investigate the development of IFC in containerized *B. nigra* from different Iowa provenances compared to the industry standards, so, we chose to evaluate provenances of *B. nigra* occurring from the far west-central distribution to an elevated pH substrate, as they may possess much better pH adaptability than those occurring in the southeast and the trade standards 'Cully'. Additionally, these provenances may be more cold hardy than the marginally-hardy trade standard 'BNTMF'. Foliar nutrient and chlorophyll (a and b) contents, along with plant growth characteristics were measured to determine each sources response to an elevated pH substrate.

Materials and Methods

Source Materials

Expt. 1

Two experiments were conducted during the course of this work. Expt. 1 (21 July 2017 to 19 Nov. 2017) screened seedlings from 11 sources of *B. nigra*. Expt. 2 (31 Aug. 2017 to 12 Dec.

2017) screened six clones of *B. nigra*, including the cultivars 'Cully and 'BNMTF', along with progeny from 'BNMTF'. Both experiments were conducted in a polycarbonate greenhouse at the Kansas State University John C. Pair Horticultural Center (Haysville, KS).

For Expt. 1., 10 seed lots from individual open- pollinated (OP) specimens of *B. nigra* occurring in five different native stands in Iowa were collected by the staff of the North Central Region Plant Introduction Station (NCRPIS) [Agricultural Research Service (ARS), United States Department of Agriculture (USDA), Ames, IA] in June 2014 (Table 3.1). Another source was collected from a mature residential specimen of OP *B. nigra* 'BNMTF' on 21 May 2017 near Wichita, KS.

All seed collected by the NCRPIS (5 and 6 June 2014) were stored at -18 °C. Seeds were received on 15 Dec. 2016 and stratified for 36 days at 3 °C by placing each accession between two 27.94 cm x 13.97 cm of sheets wetted paper towel in a 0.65 L closed polyethylene bag. After stratification, seeds for each accession were sown in flats (2400B Heavy Duty Portland Flat, Anderson Pots Inc., Portland, OR.) on the surface of soilless media (Metro- Mix 360, Sun Gro Hortuclture, Agawam, MA), covered with 0.42 cm of silica sand (Natural Play Sand, Pavestone, Atlanta, GA.), and watered. Flats were hand-misted once daily with tap water to prevent desiccation. One week after sowing, flats received a drench of (R,S)-2-[(2,6-dimethylphenyl)-methoxyacetylamino]-propionic acid methyl ester (mefenoxam) [0.13 $mL \cdot L^{-1}$ reverse osmosis (RO) water, Syngenta® Greensboro, NC]. Within 2 weeks of sowing, uniform germination was observed. On 21 May 2017 (59 days after sowing), seedlings 2.5 to 3.8 cm tall were moved to a porous pine bark substrate (Yardcare™ Small Western Bark, Mountain West Products, Rexburg, ID.) in 3.8 cm x 18.4 cm cone trainers (SC10RA, Stuewe and Sons, Inc., Tangent, OR.) under hourly overhead mist irrigation with RO water from 8:00 AM to 6:00 PM. Seeds of OP residential

B. nigra ‘BNMTF’ (21 May 2017 collection) were air dried at 25 ℃ for 2 days, and stratified for 30 days following the same procedure stated above. Seeds were sown and grown in flats for 2 weeks using the same materials and procedure as described above. After germination, all seedlings were fertilized once weekly with 200 ppm N [Jack’s Professional (20N–1.3P–15.7K)].

On 7 July, 2017, 25 seedlings per accession where transferred to 6 cm x 6 cm x 9.5 cm (0.29 L) square containers (AB34, Anderson Pots, Portland, OR.) with 1:1 (v:v) soil conditioner (Yardcare™ Soil PEP, Mountain West Products, Rexburg, ID.) : sphagnum peat moss (Fertilome Sphagnum Peat Moss, Voluntary Purchasing Groups Inc, Bonham, TX) amended with 2.8 $kg \cdot m^{-3}$ Osmocote 14N-4.2P-11.6K (Everris NA, Inc., Dublin, OH), and 0.5 $kg \cdot m^{-3}$ Micromax® (Everris NA, Inc., Dublin, OH) and and 0 $kg \cdot m^{-3}$ (control) or 11.9 $kg \cdot m^{-3}$ (elevated) of dolomitic lime (Deco® Lawnlime®, The Georgia Marble Co., Kennesaw, GA). Codominant leaders and auxiliary shoot growth was removed weekly so each seedling retained one central leader. Seedlings acclimated to new substrate for 2 weeks substrate before treatment drench initiation.

Expt. 2

For Expt. 2, seven sources of *B. nigra* were used. Five taxa were clones obtained by rooting hardwood cuttings from single trees growing at the John C. Pair Horticultural Center, Haysville, KS. Four of those five originated from individual OP *B. nigra* in Iowa collected by the NCRPIS in June 2014 (Table 3.2). The fifth clone obtained by hardwood cuttings was *B. nigra* ‘BNMTF’ located in the same field trial as the Iowa sources. *B. nigra* ‘Cully’ (1.5 m tall) in 3.8 L containers were obtained from a wholesale grower (Greenleaf Nursery, Park Hill, OK). The seventh source were seedlings collected in May 2016 from the same OP *B. nigra* ‘BNMTF’ in Expt. 1 grown in band containers (AB46, Anderson Pots, Portland, OR.) with soilless media (Metro- Mix 360, Sun Gro Hortuclture, Agawam, MA) until the rooted cuttings from the Iowa

sources and OP *B. nigra* 'BMNTF' were of appropriate size for transplantation. All plants were potted into 3.8 L containers (Classic 400, Nursery Supplies Inc.™, Chambersburg, PA) filled with the same substrate described in Expt. 1.

Design and Treatments

Expt. 1

Expt. 1. Beginning 21 July 2017 plants were irrigated twice weekly with 120 mL solution of 0 or 4.77% liquid calcium (CalOx® pH, BioSafe Systems LLC., Hartford, CT) and 0.122% magnesium sulfate heptahydrate [($MgSO_4 \cdot 7H_2O$), PDC Brands™ Stamford, CT) to create a low (control) and elevated pH substrate, respectively. Fifty-eight days after treatment initiation, plants were transplanted into 9.2 cm x 15.2 cm (2.83 L) band containers (AB46, Anderson Pots, Portland, OR.) with 1:1 (by vol.) 1.27 cm screened pine bark : sphagnum peat moss (Fertilome Sphagnum Peat Moss, Voluntary Purchasing Groups Inc, Bonham, TX) amended with 2.8 $kg \cdot m^{-3}$ Osmocote 144.211.6 (Everris NA, Inc., Dublin, OH), 0.5 $kg \cdot m^{-3}$ Micromax® (Everris NA, Inc., Dublin, OH), and 0 $kg \cdot m^{-3}$ (control) or 11.9 $kg \cdot m^{-3}$ (elevated) of dolomitic lime (Deco® Lawnlime®, The Georgia Marble Co., Kennesaw, GA). Prior to potting, all media was washed from the roots with tap water. One week after potting, treatment drench applications continued with either 120 mL solution of 0% (control) or 4.77% liquid $CaCO_3$ (elevated).

Plants were grown on a greenhouse bench in a polycarbonate greenhouse under 50% shade cloth. Mean photosynthetic photon flux was 224 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (n = 17) measured at mid-day with a quantum meter (QSW-01, Apogee Instruments, Inc., Logan, UT). To increase photosynthetically active radiation shade cloth was removed 70 d (Expt. 1) and 30 d (Expt. 2) after study initiations. Mean photosynthetic photon flux after shade cloth removal was 624 $\mu mol\ m^{-2} \cdot s^{-1}$ (n = 20) at mid-day. Plants were exposed to supplemental lighting the remaining 44 (Expt.

1) and 67 d (Expt. 2) after study initiation four 400 watt high pressure sodium (HPS) and two 400 watt metal halide lights. Lights were suspended 2.4 m above plants and operated daily from 6:00 AM–12:00 PM (7h 00 min); 4:00 PM –12:00 AM (8h 00 min). Greenhouse temperatures were set to 24 °C/20 °C (day/night).

Substrate solution pH and EC were determined using the pour-through technique (Wright, 1986). Values were measured four times over the span of the experiment. Chlorophyll content was measured indirectly using a SPAD meter (SPAD-502, Konica Minolta, Inc.) and directly following the methods of Porra et al., (1989). SPAD ratings were taken after 33 d and 119 d, (23 Aug. and 17 Nov. 2017, respectively). Average SPAD ratings were collected by recording one data point from one location on the lower-right from the midrib on three most recently, fully expanded leaves per plant. For direct chlorophyll measurement, combined replications were used to ensure sufficient leaf tissue per treatment. Leaf chlorophylls a and b were obtained by punching 16 mm^2 leaf discs from four of the most fully expanded leaves per plant the day before experiment termination. Leaf discs were taken from the bottom right side per leaf. Eight leaf discs (four per replication) were placed in a 56 mL glass test tube with 16 mL *N*,*N*-Dimethylformamide (DMF) (Fisher Scientific, Hampton, NH). Chlorophylls were extracted under dark conditions for 24 hr. During the last 12 hr of extraction, test tubes were placed on an orbital shaker (Innova 2100, New Brunswick Scientific, Edison, NJ) at 80 rpm and 24 °C. Before spectrophotometric analysis, all samples were homogenized at 860 rpm for 2 s (Vortex Genie 2, Fisher Scientific, Hampton, NH). Absorbance at 647 and 664 nm, was determined using spectrophotometric analysis with a Hitachi U1100 spectrophotometer (Hitachi, Ltd., Tokyo, Japan). Leaf chlorophyll a and b concentration was determined using the equation derived by Porra et al., (1989).

Approximately 17 weeks after treatment initiation (19 Nov. 2017), the experiment was terminated and additional plant growth and development parameters were collected. Data included plant height, stem caliper at substrate interface, leaf number, leaf size, and dry weight of stem and leaf tissue. Total leaf area per plant was determined using a leaf area meter (Model Li-3100C, Li-Cor®, Lincoln, NE). Dry weights were determined by placing plant tissue in paper bags in a forced-air drying oven (Grieve SC-350 Electric Shelf Oven, Round Lake, IL) at 71°C for 7 d. Total carbon (C) and nitrogen (N) in leaf tissue on the most recently matured leaf tissue were obtained with a C/N combustion analyzer (LECO TruSpec CN, LECO Corporation, St. Joseph, MI) by the Kansas State Soil Testing Laboratory (Kansas State University, Manhattan, KS). Furthermore, phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), Zn (Zinc), Fe, Cu (Copper), and Mn (Manganese) in leaf tissue was obtained from Perchloric digest (Inductively Coupled Plasma (ICP) Spectrometer, Model 720-ES ICP Optical Emission Spectrometer, Varian Australia Pty Ltd., Mulgrave, Vic. Australia) (Gieseking et al., 1935).

The experimental design was a randomized complete block with a factorial arrangement of treatments (seed source x substrate pH). There were eleven seed sources in combination with two substrate pH levels creating twenty-two treatments. Each treatment combination was replicated eight times. However, similar treatments from different replications were combined to ensure sufficient tissue for some analysis procedures. Therefore, replications were combined for direct chlorophyll determination and leaf nutrient analyses (four combined replications per seed source x substrate pH), except three for PWMA1, PWMA2, BSP1, BSP2, BSP3, and CF2 x elevated pH substrate. For final SPAD, each (accession x substrate pH) had eight replications except only seven in the elevated treatments for PWMA1, PWMA2, and BSP3, and only six for BSP1, BSP2, and CF2. Data were subjected to an analysis of variance using proc GLM (SAS

Institute Inc., Cary, NC). When appropriate, means were separated using the lsmeans option with Fisher's Protected LSD $P \leq 0.05$.

Expt. 2

Expt. 2. On 31 Aug. 2017, after 14 weeks of growth, all seedlings growing in 3.8 L containers, were removed and root systems were washed with tap water, then potted into 11.4 L containers (C1200, Nursery Supplies Inc., Chambersburg, PA) using the same substrate and rates of amendments as described when seedlings were potted on 21 July in Expt. 1. Containers were placed on a gravel surface in the same polycarbonate greenhouse under the same environmental conditions as Expt. 1. One week after planting, all trees were pruned to 15.24 cm of stem height. Two weeks after pruning, twice-weekly irrigations with 360 mL of 0 or 4.77% liquid $CaCO_3$ solution (CalOx® pH, BioSafe Systems LLC., Hartford, CT) with 0.122% magnesium sulfate heptahydrate [($MgSO_4 \cdot 7H_2O$), PDC Brands™ Stamford, CT) in tap water (similar to Expt. 1) were applied to each container and continued to experiment termination. Over the course of the experiment, substrate solution pH and EC were monitored five times using the pour-through technique.

Fifteen weeks after planting (12 Dec. 2017) data were collected and the experiment was terminated. Data collected included final SPAD, substrate pH and EC, and leaf chlorophyll a and b concentration. Plant growth data included plant height, stem caliper at soil interface, leaf number, leaf size, and dry weight of stem and leaf tissue, and leaf tissue nutrient analysis following the same procedures as Expt. 1.

The experimental design was a randomized complete block with a factorial arrangement of treatments (source x substrate pH). There were seven sources in combination with two pH levels creating 14 treatments. Each treatment combination was originally replicated 8 times, but

less replications were used due to poor uniformity resulting from the lack of auxiliary bud break after pruning; Table 3.3 shows the number replications of per treatment. Data were subjected to an analysis of variance using proc GLM (SAS Institute Inc., Cary, NC). When appropriate, means were separated using the lsmeans option with Fisher's Protected LSD $P \leq 0.05$.

Mouse ear disorder and pest control

Before initiation of treatments, mouse ear disorder (MED) was observed on a few plants grown from cuttings. A foliar application of 400 ppm nickel (II) sulfate hexahydrate (99.9% $NiSO_4 \cdot 6H_20$, Loudwolf Industrial Scientific, Dublin, CA) with 1.06 $mL \cdot L^{-1}$ water of a non-ionic surfactant (Polyoxyethylene (20) sorbitan monolaurate, Fisher Scientific, Fair Lawn, NJ.) in RO water was applied to plants in both experiments. Visual symptoms of nickel deficiency thereafter were not observed. During the experiments pest pressure was very low, only requiring two insecticides applications for aphids (*Melanaphis sacchari* Zehntner). Control was achieved with foliar sprays of azadirachtin (0.62 mL 4.5% azadirachtin $\cdot L^{-1}$ RO water, Certis USA, Columbia, MD) and pymetrozine (1.93 g 50% pymetrozine $\cdot L^{-1}$ RO water, Syngenta® Greensboro, NC). Furthermore, a soil surface spray for fungus gnats (*Bradysia* spp.) with spinosyn A and D (0.8 mL 11.6% spinosid $\cdot L^{-1}$ RO water, Dow AgroSciences™, Indianapolis, IN) as applied.

Results

Expt. 1

For Expt. 1, pH and leachate trends are shown in Figure 3.1A and 3.1B respectively. Mean leachate pH for the elevated substrate (pH = 7.57) remained high over the sampling period (pH = 7.23, 7.53, 7.70, and 7.79 after 14, 87, 101, and 114 d, respectively). Mean leachate pH for the control pH substrate (pH = 5.76) gradually increased over the sampling period (pH = 5.28, 5.26, 5.90, and 6.59 after 14, 87, 101, 114 d, respectively. The elevated pH substrate was

significantly higher compared to the control pH substrate within sampling date (all comparisons $P < 0.0001$). Mean leachate EC for control and elevated pH substrates increased between 14 and 114 DAP but was dramatically lower 114 DAP. Upon termination, leachate EC values between substrate treatments were not statistically different at $P \leq 0.05$ (control = 1.35 and elevated =1.32 $mS \cdot cm^{-1}$, $P = 0.8178$).

Significant interactions between main effects (substrate pH and seed source) were observed for plant height, stem diameter, number of leaves, and leaf and shoot dry weights (Table 3.4). In Table 3.5, non-significant increases were observed for plant height were observed for some seed sources (6.9%, PWMA1 and 6.6% OP 'BNMTF') growing in the elevated pH substrate, while in other seed sources, non-significant decreases in plant height (between 1.9 – 11% for PWMA3, PWMA2, CF1, and, CCWMA2) compared to their controls were observed. The remaining seed sources experienced significant reductions in height by 19% for BSP30, 21% CF2, 27% CF1, and 30% BSP1. Furthermore shown in Table 3.5, seedlings in the elevated pH substrate experienced non-significant decreases in seedling stem diameter between 4.0 – 11% were observed for PWMA2, BSP2, BPS3, and CWMA2 and others experienced significant decreases compared to their respective controls 12% for CCWMA1 and OP 'BNMTF', 15% PWMA1, 17% CF1 and CF2, 18% BSP1, and 30% PWMA3. We observed non-significant increases in leaf number (5.2%, PWMA2), along with non-significant reductions (12 to 28% for CCWMA1, CCWMA2, PWMA3, CF1, and CF2), and significant reductions (30% for BSP3, 36% BSP1, 41% OP 'BNMTF', 45% PWMA1, and 51% BSP2) for seed sources growing in the elevated pH substrate compared to their controls. Similar trends for total leaf and stem dry weights were observed (Table 3.5). In the elevated pH substrate, CF1, PWMA2, BSP3, and PWMA1 had insignificant reductions in total leaf and shoot dry weights compared to their

controls, 8.9% to 18% and 0.57 to 24%, respectively while others were greatly reduced in leaf (first percentage) and shoot (second percentage) dry weights [(45% and 36%, CF2), (52% and 36%, BSP1), and (59% and 50 %, BSP2)]. Seedlings grown in the elevated pH substrate were reduced in leaf area (1215.5 cm^2) compared to the control (1847.0 cm^2) where CCWMA1 (1860.2 cm^2) had the greatest leaf area, but not statistically greater than OP 'BNMTF' (1848.1 cm^2), PWMA2 (1769.6 cm^2), and CCWMA2 (1632.9 cm^2) (all seed source means for total leaf area not shown).

After 33 days, no difference in leaf SPAD between substrate treatments was found (control = 36.70 and elevated = 36.44, $P = 0.7057$), and all plants were symptomless of IFC, but differences in seed source were apparent ($P < 0.0001$). OP 'BNMTF' had statistically lower SPAD ratings (27.31) compared to the Iowa seed sources (ranging from 34.88 to 39.25). Just before termination at 119 DAP, a marginally significant interaction between seed sources and substrate pH were found to affect SPAD values (Table 3.4 and 3.7). Even though all sources growing in the elevated pH substrate had lower SPAD readings respective to their controls, leaf SPAD for BSP3 and CCWMA1 was only reduced by 17 and 19%, respectively, while other sources such as BSP1, OP 'BNMTF', and PWMA1 were reduced by much greater proportions (43, 46, and 55%, respectively). In addition to measuring SPAD, leaf chlorophyll a and b concentrations were measured, as an additional parameter to further quantify the IFC development. Seed source and substrate treatments both affected leaf chlorophyll concentration but their interaction was not significant (Table 3.4). Seedlings grown in the control pH substrate had approximately 10 $\mu g \cdot cm^{-2}$ (36%) more leaf chlorophyll than plants grown in the elevated pH substrate (Table 3.6). Overall, seed source BSP3 had the greatest leaf chlorophyll content, although not statistically greater than CCWMA1 or CCWMA2. The seed sources with the lowest

leaf chlorophyll content were OP 'BNMTF' and PWMA1, although not statistically different than many of the Iowa seed sources (BSP1, BSP2, CF1, CF2, PWMA2, and PWMA3).

The micronutrient concentrations of leaves relative to seed source and substrate treatments when no interaction was observed are shown in Table 3.6. A substrate treatment effect but no seed source effect was observed for total leaf Fe and Cu contents where both of these micronutrients were reduced by 61% in the elevated pH substrate. Marginal interactions between main effects were observed for total leaf Mn (Table 3.4). In Table 3.7, large reductions in total leaf Mn and for all seed sources in elevated pH substrates compared their controls can be observed (between 90 and 94%); where in the control pH substrate, BSP2 sequestered more Mn than the other treatments expect CF also in the control pH substrate, and all seed sources in the elevated pH substrate sequestered equal leaf Mn content. No significant interaction was observed between main effects for leaf Mn:Fe ($mg \cdot kg^{-1}$: $mg \cdot kg^{-1}$), but the substrate treatment ($P < 0.0001$) and seed source effects were significant ($P < 0.0223$). Control plants had much greater Mn:Fe values than the elevated pH substrate and seed source BSP3 had the greatest Mn:Fe, although not statistically higher than CF1 and PWMA1 (Table 3.6). Other than leaf Mn content, an interaction between main effects was observed for total leaf Zn where reductions for a seed source in the elevated pH substrate compared to their controls were less dramatic (between 33% and 62%), and once again, we observed BSP2 sequestering greater leaf Zn compared to the other treatments (Table 3.7).

The macronutrient concentrations of leaves relative to the substrate pH treatments and seed sources are shown in Table 3.8. No statistical difference for leaf N content between substrate treatments was found, but seed source significantly affected leaf N content. OP 'BNMTF' had the greatest leaf N content per kg of dry weight (not statistically greater than

PWMA2), where CF2 had the lowest (but only statistically lower than OP 'BNMTF', BSP3, PWMA2, and PWMA3). Substrate treatment did not affect leaf P per kg of dry weight, but the interaction between seed source and substrate treatment was significant (Table 3.4). Increases in total leaf P by 7, 8, and 43% were observed for OP 'BNMTF', PWMA2, and BSP1 seedlings, respectively, growing in the elevated pH substrates compared to their controls, these three seed sources had the lowest leaf chlorophyll contents (but only significantly lower than BSP3) (Tables 3.6 and 3.8). While BSP3 had lower leaf P contents per kg of leaf dry weight when compared to OP 'BNMTF', PWMA2, and BSP1 in the elevated pH substrates, it did not have statistically lower a P:Fe (%:%) than the three other seed sources. Reductions of total leaf P per kg of leaf dry weight by 26 and 29%, respectively, were observed for BSP3 and CCWMA2, and where only marginal reductions for sources in the elevated pH substrate existed compared to their controls (1 – 9%) (Table 3.7). The trend of total leaf Fe and Cu depression previously seen for the seedlings growing in the elevated pH substrate were opposite for total leaf K, Ca, and Mg. Leaf K, Ca, and Mg contents per kg of leaf dry weight were statistically higher in the elevated pH substrate treatment plants (Tables 3.4 and 3.8). Furthermore, seed source affected total leaf K content. OP 'BNMTF' had greater leaf K per kg of leaf dry weight than the seed sources with high chlorophyll contents: BSP3 ($P = 0.0052$) and CCWMA1 ($P < 0.0001$)].

Expt. 2

For Expt. 2, pH and leachate trends are shown in Figure 3.2. Mean leachate pH (pH = 7.00 over 91 d) for the elevated substrate treatment began high (7.17 after 14 d), but significantly fell after the first sampling date (6.71 after 28 d) ($P = 0.0137$). The $CaCO_3$ applications raised the pH for elevated substrate treatment on subsequent sampling dates after 56, 77, and 91 d was : 7.06, 7.08, and 7.07, respectively. The pH for the control pH substrate treatment (pH = 5.29 over

91 d) after 14, 28, 56, 77, and 91 d was: 5.23, 5.18, 5.43, 5.41, and 5.16, respectively. Despite the initial drop in leachate pH in the elevated pH substrate, mean leachate pH values for the control pH substrate treatment was always lower than the elevated pH substrate at $P \leq 0.05$ (all comparisons $P < 0.0001$). An increase in mean leachate EC for the substrate treatments was observed over the course of the experiment. Leachate EC values between substrate treatments were statistically different at $P \leq 0.05$ after 77 d (control = 4.88 and elevated = 6.32 $mS \cdot cm^{-1}$, $P < 0.0001$) and after 91 d (control = 5.23 and elevated = 6.12 $mS \cdot cm^{-1}$, $P = 0.0088$).

Similar to Expt. 1, all plant growth characteristics were affected by substrate treatment and source, but their interactions for a growth characteristic were not observed (Table 3.9). Plants grown in the elevated pH substrate treatment had reduced mean stem length, mean stem diameter, number of leaves, total leaf area, and leaf and shoot dry weights (Table 3.10). Source CCWMA2, had the greatest mean stem length, mean stem diameter, total leaf area, and leaf dry weight (although mean stem length and diameter for CCWMA2 was not statistically greater than PWMA2).

After 103 d and upon experiment termination, leaf SPAD ratings had been affected by substrate pH and source (Table 3.9). Leaf SPAD was greater in the control pH substrate treatment (control = 36.44 and elevated = 24.67) ($P < 0.0001$) (Table 3.10) and many plants in the elevated pH substrate treatment showed symptoms IFC. Source CCWMA3 had the greatest leaf SPAD readings followed by 'BNMTF' (not statistically different), CCWMA2, PWMA2, and CF3, while sources OP 'BNMTF' and 'Cully' had the lowest SPAD readings. Leaf chlorophyll content was also used to confirm the development of IFC; seed source and substrate treatments were found to affect leaf chlorophyll content. Seedlings in the control pH substrate had approximately 9.3 $\mu g \cdot cm^2$ (32%) more leaf chlorophyll a and b concentrations than seedlings in

the elevated substrate. The source with the greatest leaf chlorophyll content after 103 d was CCWMA3, which was not statistically higher than 'BNMTF', CF3, and PWMA2, but was 10%, 18%, and 22% statistically higher than three lowest preforming sources (CCWMA2, 'Cully', and OP 'BNMTF', respectively) (Table 3.10). Figures 3.3 and 3.4 exhibit comparisons between germplasm sources in control and elevated pH substrates and CCWMA3, 'Cully', and 'BNMTF' in elevated pH substrates.

Total leaf Fe, Mn, and Zn micronutrients were significantly affected by substrate treatment and seed source with significant interactions between the main effects also observed (Table 3.9 and Table 3.12, respectively). Even though all sources in the elevated pH substrate treatment had statistically lower leaf Fe contents than their respective controls, CF3 (39.60 $mg \cdot kg^{-1}$) had the greatest leaf Fe content compared to the other sources and was only reduced by 29% compared to its control. Total leaf Fe contents for the other sources were reduced by much greater magnitudes; 39%, CCWMA3; 45%, OP 'BNMTF'; 48%, PWMA2; 56%, 'Cully'; 61%, 'BNMTF'; and 65%, CCWMA2. In the control pH substrate, the greatest contents of total leaf Mn, were observed for 'Cully' (5059 $mg \cdot kg^{-1}$) followed by 'BNMTF' (3721 $mg \cdot kg^{-1}$) and CF3 (3212 $mg \cdot kg^{-1}$), although CF3 was not statistically greater than CCWMA3 and PWMA2. Reductions in total leaf Mn content were found in the elevated pH substrate treatment for a source compared to the controls where reductions of leaf Mn contents were observed by similar magnitudes: 84% CWMA2, 89% CCWMA3, 90% PWMA2 and 'BNMTF', 91% OP 'BNMTF', 94% CF3, and 95% Cully. A significant interaction ($P < 0.0001$) was observed between substrate treatment and seed source for leaf Mn:Fe (both $P < 0.0001$).'Cully' had a greater Fe:Mn in the control pH substrate had than the other treatments, and within each seed source, Mn:Fe was always reduced in elevated pH substrate compared to its respective control (Table 3.12). We

observed a similar trend with total leaf Zn contents. In the control pH substrate 'Cully' (478 $mg \cdot kg^{-1}$) had the greatest total leaf Zn content where 'OP 'BNMTF' (250 $mg \cdot kg^{-1}$) had the least. In the elevated pH substrate, reductions in total leaf Zn content were found between within a source where they were all significantly reduced: 47% CWMA2, 50% PWMA2, 52% OP 'BNMTF', 56% CWMA3, 58% 'BNMTF', 60% CF3, and 65% Cully. Similar to Expt. 1, total leaf Cu was only affected by the substrate pH where it was reduced by 79% (Table 3.11).

P values for effects of substrate pH, source, and their interactions on leaf macronutrient concentrations are shown in Table 3.9. Macronutrients concentrations N, K, Ca, and Mg of per kg of leaf dry weight for source and substrate pH are shown in Table 3.11. Total leaf N was statistically reduced in the elevated pH substrate by 6.9% where total leaf K, Ca, and Mg contents increased by 14%, 24%, and 23%, respectively (Table 3.11). Additionally, seed source affected total leaf N, Ca, and Mg contents (Table 3.9). Total leaf N content was greatest in the two trade cultivars: 'Cully' (3.65%) and 'BNMTF' (3.60%) although, leaf N contents in 'BNMTF' were not statistically greater than PWMA2 or CCWMA2 (Table 3.11). In addition, total leaf Ca content was the greatest in 'Cully' (1.50%), but not statistically greater than seed sources PWMA2 (1.40%) and CF3 (1.32%). Moreover, total leaf Mg content was greatest in 'Cully' (0.342%) and 'BNMTF' (0.341%), but both were neither statistically greater than CF3 (0.335%) and PWMA2 (0.332%). Unlike the other macronutrients, an interaction between the main effects was observed for total leaf P (Table 3.9). 'Cully" had the highest leaf P in both the control and elevated pH substrates compared to the other treatments (Table 3.12). In the elevated pH substrate, increases in total leaf P for 'BNMTF' (11%) and OP 'BNMTF' (21%) occurred, while total leaf P decreased for the other sources: 1.2% CF3, 10% PWMA2, 11% 'Cully', 12% CCWMA2, and 30 % CCWMA3. Furthermore, we observed a significant interaction between

substrate treatment and seed source effects for leaf P:Fe (Table 3.9). Shown in Table 3.12, 'Cully', CF3, and OP 'BNMTF' in the elevated pH substrate had a greatest P:Fe had than the other treatments, and within each seed source, P:Fe was always increased in elevated pH substrate compared to its respective control.

Discussion and Conclusions

In these studies, we investigated IFC development in Iowa provenances and trade standards of *B. nigra* growing in elevated substrate pH, based on reports that *B. nigra* develops IFC in the landscape from the lack of Fe availability in alkaline soils (Bartlett, 2015; Carlson, 2003; Whitman and Ranney, 1994 and 1995; McNamara and Pellet, 2001).

In Expt. 1 for all growth characteristics expect total leaf area, we observed interactions between our main effects (substrate pH and seed sources) where the elevated pH substrate impacted growth characteristics for the seed sources differently compared to their controls. No reductions in growth characteristics were observed for PWMA2; while for others, like BSP1, all growth characteristics were statistically reduced. In Expt. 1, we must acknowledge that differences in growth characteristics in a seedlings response to high pH varied within a seed source, because these seed sources from Iowa used were collected from OP parents within different populations. Clausen (1966) suggested that *B. nigra* is nearly self-incompatible and heavily relies on intraspecific hybridization to maintain heterozygosity and fitness within populations thus progeny from a single tree may widely vary in growth traits from the populations he observed. In Expt. 2, main effects for all growth characteristics were significant, but we did not observe significant interactions between the two at $P \leq 0.05$ as was observed in Expt. 1. All sources grew best in the control pH substrate where, overall, in the elevated pH substrate, CCWMA2 out preformed OP 'BNMTF' and 'Cully' but eventually developed IFC.

Furthermore, the growth measurements for the all cloned sources and the one seed source did not always substantiate the differences in IFC between sources like SPAD and leaf chlorophyll. All source material for Expt. 2 was obtained from a variety of sources; 'BNMTF' and Iowa clones originated as hardwood field cuttings from single trees, OP 'BNMTF' was grown from seed, and 'Cully' originated from wholesale nursery stock. Even though, all material was decapitated a week after experiment initiation with the intent to homogenize growth responses, differences between source material and number of latent buds left on the shoot most likely effected the their growth response and vigor.

Leaf SPAD readings [(Table 3.7, Expt. 1) and (Table 3.10, Expt. 2)] and leaf chlorophyll a and b concentrations [(Table 3.6, Expt. 1) and (Table 3.10, Expt. 2)] quantified leaf health and confirmed IFC development. As expected, the industry standard, 'Cully', and OP 'BNMTF' developed IFC and had considerably lower leaf chlorophyll a and b concentrations when compared to the other sources. Even though 'BNMTF' had fairly equitable leaf chlorophyll a and b contents compared to the other sources (Table 3.10; Figures 3.3 and 3.4). The degree of IFC exhibited by OP 'BNMTF' in elevated pH substrate may indicate that the progeny of 'BNMTF' are poorly adapted to alkaline conditions, but again, we must acknowledge the existence of seedling variation between progeny of 'BNMTF', and the progeny are not representative of the trade standard 'BNMTF' (Clausen, 1996; Koevenig, 1976). In addition, many of the Iowa seed sources in the elevated pH substrate developed IFC and did not maintain enough leaf chlorophyll a and b to resist IFC. At the end of both experiments, all plant materials grown in the elevated pH substrate had developed varying severities of IFC; some more significant than others. A couple native Iowa sources may likely be good candidates to further investigate for tolerance to IFC [(Expt. 1: BSP3 and CCWMA1 and CCWMA2) and (Expt. 2: CCWMA3)] based on SPAD

ratings and leaf chlorophyll a and b concentrations observed in our studies. From the soil and nutrient data collected by NCRPIS ARS, USDA, Ames, IA, high pH and high Ca content soil was not necessarily indicative for a provenances tolerance to IFC. A soil test from Bearbower Sand Prairie, Buchanan Co., IA for seed source BSP3 indicated a soil profile with a moderately acidic pH and low calcium content, but BSP3 was one of the better preforming seed sources. This may indicate that the selection of *B. nigra* from seed sources derived from high pH soils and calcareous soils may not always hold true. Furthermore since BSP1 and BSP2 did not perform as well as BSP3, this may suggest genetic variation within the population.

Total leaf Fe, Mn, Cu, and Zn concentrations of seedlings were severely depressed in the elevated substrate pH treatments which possibly caused nutritional constraints, potentially limiting healthy plant growth and development, as these micronutrients are important for protein and hormone synthesis, enzyme activation, fatty acid production, used in redox reactions for photosynthesis, and for chlorophyll formation (James et al., 2008; Livorness 1982; Marschner, 2011; Waters and Armburst, 2013). We did not measure the available substrate fraction of these micronutrients, in which those data could provide support to the claim that as pH and $CaCO_3$ concentration of a medium increases, the availability of these nutrients for plant uptake decreases (Chatizanthis et al., 2014; Mengel and Kirkby, 2001; Marschner, 2011; Römheld and Marschner, 1986).

In Expt. 1, the lack of statistical variation between seed sources for total leaf Fe content may suggest none of these sources are any more capable than the other of acquiring substrate Fe at high pH, but since differences in leaf chlorophyll a and b concentrations were observed between seed sources, BSP3 may have a greater Fe use efficiency than all but CCWMA1 and CCWMA2 (Kobayashi and Nishizawa, 2012; Martinez-Cuena and Primo-Capella, 2017; Waters

and Troupe 2012). In Expt. 2, total leaf Fe was not indicative for the amount of IFC observed, which is reasonable considering total Fe is not indicative to amount of physiologically active (Fe^{+2}) used for chlorophyll formation (Katyal and Sharma, 1980; Nikolic and Römheld, 2002). From field grown trees of *B. nigra*, total leaf Fe had been reported to range from 43.0 to 233 $mg \cdot kg^{-1}$ where we observed chlorotic plants having no more than 39.6 $mg \cdot kg^{-1}$ (Mills and Benton, 1996), but source CCWMA3, which was less chlorotic, had 27.1 $mg \cdot kg^{-1}$ total leaf Fe.

Under Fe-deficiency, IRON-REGULATED TRANSPORTER 1 (IRT1) is primarily responsible for the transportation of Fe into the root from the rhizosphere during the Strategy I response, but it can actively transport zinc and other divalent cations too (Cohen et al., 1998; Vert et al., 2002). Species of *Betula* have not yet been directly observed using the Strategy I Fe-acquisition mechanism. Furthermore, Zn can be accumulated in excess under Fe deficiency (Kanai et al., 2009). We did not observe excess foliar Zn accumulation in both experiments, which may suggest that it was less available for root uptake because of its pH dependency. Furthermore, we did not observe leaf Zn deficiencies in *B. nigra* sources with IFC. A normal range of leaf Zn is anywhere between 23 and 212 $mg \cdot kg^{-1}$ for field grown plants while we observed leaf Zn contents between 83 and 167 $mg \cdot kg^{-1}$ *B. nigra* with IFC; generally we observed greater leaf Zn contents in healthy leaves than what was previously reported (Mills and Benton, 1996). In addition to Zn, since total leaf Cu was reduced at high pH, optimal ferric chelate reductase (FCR) activity, a crucial step in the Strategy I response for Fe deficiency may have been inhibited (Marschner, 2011; Waters and Armbrust, 2013). If species of *Betula* do indeed use the Strategy I Fe- acquisition mechanisms before Fe uptake, low plant supply of Cu would have depressed optimal FCR activity. A normal sufficiency range of total leaf Cu in field grown *B. nigra* is between 4.0 to 11 $mg \cdot kg^{-1}$ (Mills and Benton, 1996) where we observed much lesser

foliar contents for seedlings growing in the elevated pH substrate (2.57, Expt. 1 and 0.854 $mg \cdot kg^{-1}$, Expt. 2). Normally, symptoms of Cu and Zn deficiencies are not believed to be associated with IFC in *B. nigra*, but Cu and Zn deficiencies have been associated with major reductions in growth and oxidative cellular damage in various woody and agronomic crops (Dickey, 1965; Walker and Loneragan. 1981; Yu and Rengel, 1999; Zhang and Kirkham 1994). Furthermore, we observed less leaf Fe and Mn accumulation when *B. nigra* Iowa seed sources and trade standards grew in the elevated pH substrate. Our findings coincide with what McNamara and Pellett (2001) observed: OP *B. nigra* accumulated less leaf Fe and Mn when irrigated with buffered alkaline water treatments; they had not reported on total statuses for other leaf nutrients. In addition, we observed smaller leaf Mn:Fe ratios in seedlings growing in the elevated pH substrates suggesting total leaf Mn sequestration is more greatly impacted at a higher pH than Fe with the substrates and plant materials we used. Normal ranges Mn from healthy leaves widely varies from field grown *B. nigra* (29 to 1345 $mg \cdot kg^{-1}$), and we did not observe leaf Mn contents less than those of field grown *B. nigra* in chlorotic leaves from our experiments (45.5 to 366 $mg \cdot kg^{-1}$).

A trend of increased total leaf P per kg of leaf dry weight occurred for some of the sources that developed dramatic symptoms of IFC (‘Cully’ and OP ‘BNMTF’). Mills and Benton (1996) reported a narrow range for total leaf P contents from field grown *B. nigra* (0.13 to 0.30 % P); we observed a high leaf P:Fe compared to its control which may have exacerbated the extent of IFC observed in this source. A range of P:Fe of healthy leaves from field grown *B. nigra* was between 13:1 and 30:1; while, we observed higher ranges of P:Fe in chlorotic leaves in Expt. 1 (25:1 to 38:1, healthy and 66:1 to 101:1, chlorotic) (Welkie and Miller, 1993). In Expt. 2, we observed much lower ranges of P:Fe in leaves, but chlorotic leaves still had higher leaf

P:Fe (1:1 to 2:1, healthy and 8:1 to 16:1, chlorotic). It has been observed as leaf P:Fe increases, inorganic Fe becomes immobilized because it precipitates in leaf tissues thus becoming unavailable for chlorophyll production in garden tomato (*Solanum lycopersicum* L.) (De Kock et al., 1979). This may suggest that when leaf Fe is limited, Fe in the leaves becomes immobilized as leaf P:Fe increases or the increase in P:Fe is simply secondary to IFC. Like Cu, an adequate supply of K is thought to be critical for the Strategy I Fe deficiency response and sufficient FCR activity (Felle, 1998; Hansen et al., 2006). We detected increases in total leaf K, Ca, and Mg per kg of leaf dry weight for chlorotic plants within their reported ranges (0.69 to 1.62% K; 0.51 to 1.51% Ca; and 0.21 to 0.58% Mg) from field grown *B. nigra* for Expt. 1 and outside the reported range for K in Expt. 2. (Mills and Benton, 1996). The increase of total leaf of Ca and Mg per kg of leaf dry weight may have been caused by greater uptake since the treatment drenches contained $CaCO_3$ and $MgSO_4$ and supplied Ca and Mg in greater quantities to these treatments, or total leaf K, Ca, Mg contents were greater in the chlorotic plants, because of their increased influx into IFC leaves after the fact (López-Millán, 2000). Another explanation could simply be that since chlorotic leaves had less mass, leaf K, Ca, and Mg contents per kg of leaf dry weight were greater.

In conclusion, river birch (*Betula nigra*) a commonly planted ornamental tree taxon in Midwest landscapes develops IFC and is presumed to be Fe deficiency-induced foliar chlorosis when grown in alkaline soils. We could not determine if the lack of Fe is the sole factor of IFC in the sources of *B. nigra* that were screened because other nutrient (Mn, Cu, and Zn) concentrations in leaves were lacking too. The practice of selecting provenances adaptable to alkaline soils and tolerance to IFC has been practiced for many significant ornamental landscape taxa. Our studies investigating IFC development in Iowa provenances and trade standards of *B.*

nigra growing in elevated substrate pH suggest that even though some seed sources and genotypes from Iowa provenances in elevated substrate pH developed IFC to lesser of an extent and retained respectable concentrations leaf chlorophylls, they were unable to sequester sufficient amounts of substrate Fe, Mn, Cu, and Zn needed for continued optimal plant growth and development. Since studies in solid substrate mediums pose an issue with the allocation of these nutrients in all plant parts (root, stem, and leaves) and poorly offer the ability to substantiate the mechanisms behind the Strategy I Fe-deficiency response (H- ATPase and FCR activity), subsequent studies in nutrient solutions altered in pH with and without Fe should be conducted with the Iowa seed sources that showed promise, along with the popular industry standards 'Cully' and 'BNMTF' and cultivars touted as being tolerant to a high soil pH ('Dickinson' and 'Whit XXV').

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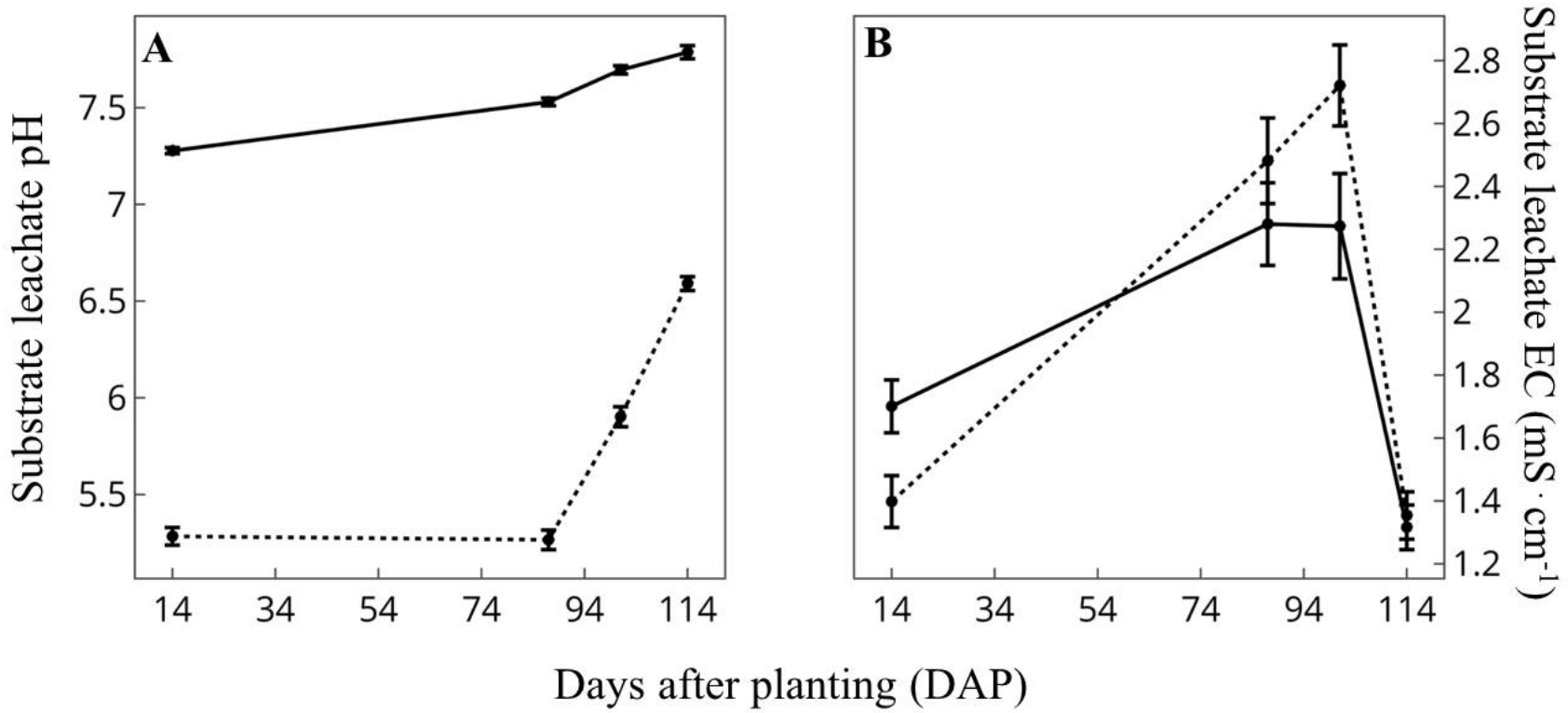

Figure 3.1 Expt. 1: Mean pour- thru leachate pH (A) and electrical conductivity (EC) (B) of *Betula nigra* seed sources from Iowa provenances and OP 'BNMTF' *B. nigra* growing in peat- bark based substrates for control (dashed line) or elevated (solid line) pH substrates. Substrates began with either 0 $kg \cdot m^{-3}$ (control) or 11.9 $kg \cdot m^{-3}$ dolomitic lime (elevated) and received either 120 mL of 0% (control) or 4.77% flowable $CaCO_3$ (elevated) twice- weekly over the course of the evaluation period. Each point represents a mean +/- SE (n =77).

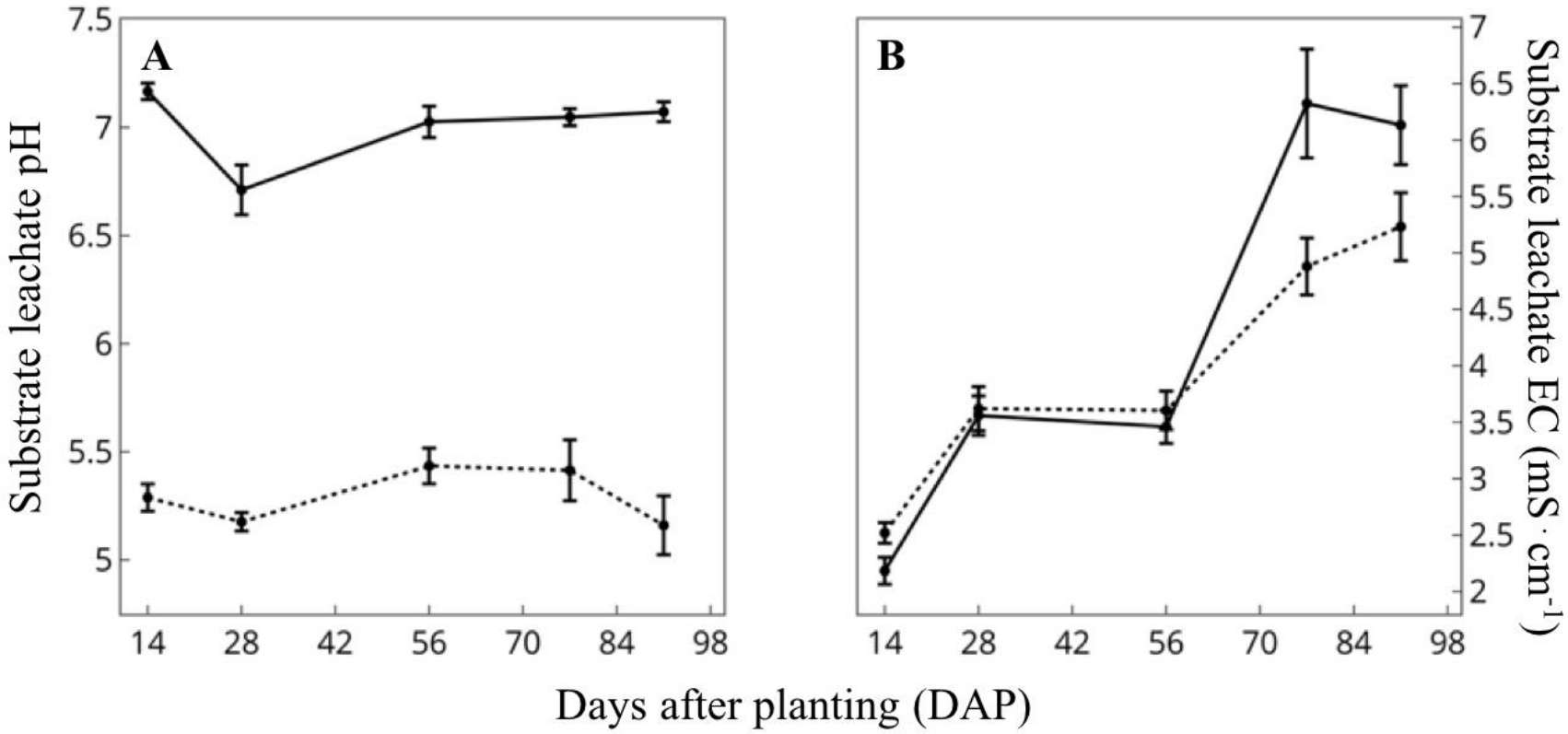

Figure 3.2 Expt. 2: Mean pour- thru leachate pH (A) and (B) electrical conductivity (EC) of *Betula nigra* clones of progeny of from Iowa seed sources, industry cultivars, and an OP 'BNMTF' growing in peat- bark based substrates for control (dashed line) or elevated (solid line) pH substrates. Substrates began with either 0 $kg \cdot m^{-3}$ (control) or 11.9 $kg \cdot m^{-3}$ dolomitic lime (elevated) and received either 120 mL of 0% (control) or 4.77% flowable $CaCO_3$ (elevated) twice- weekly over the course of the evaluation period. Each point represents a mean +/- SE (n = 45).

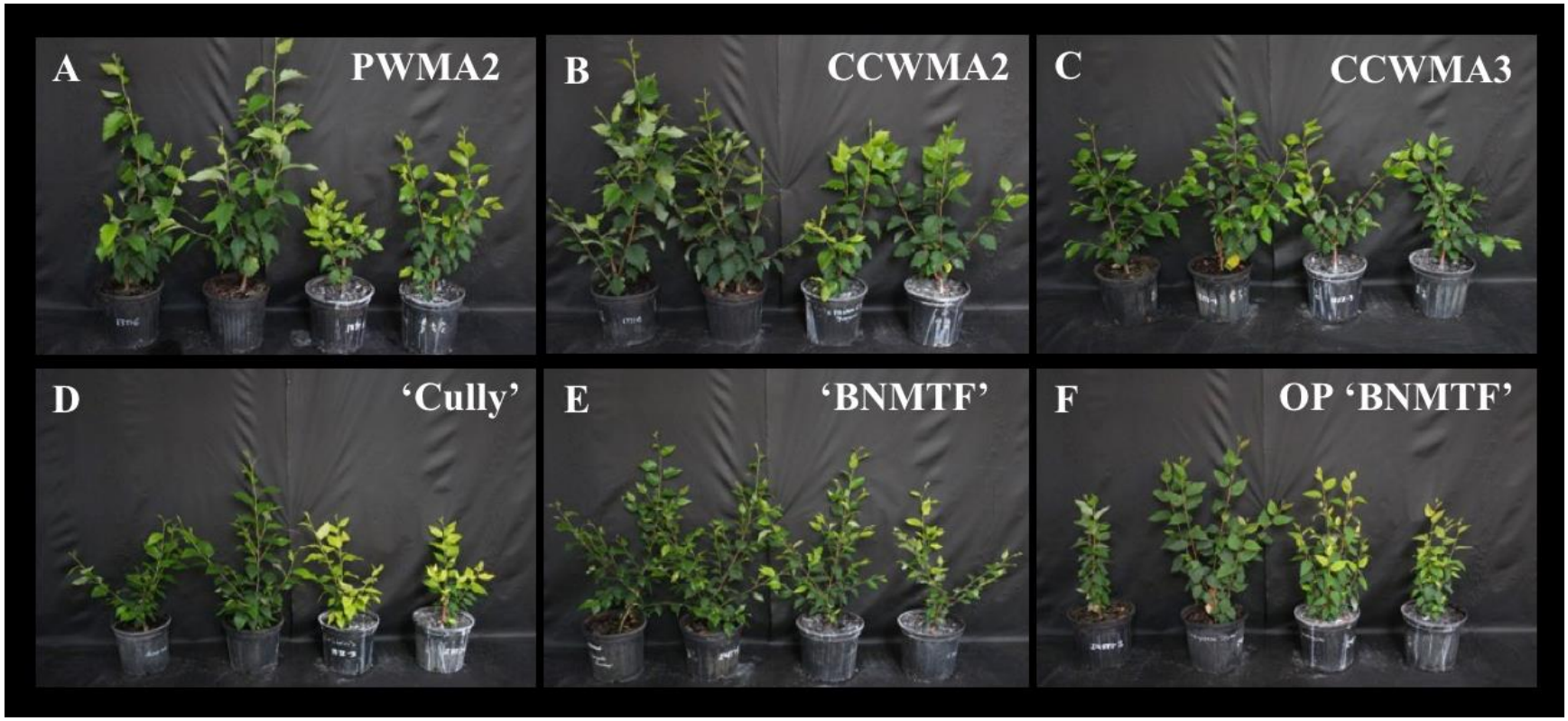

Figure 3.3 Expt. 2: each pane represents two replications per source x substrate treatment [control; pH = 5.29 (left) and elevated; pH = 7.00 (right)] Photos were taken after 101 d. Notice upper yellow foliage on stunted plants in the elevated pH substrate in panes A, B, D, and F. Furthermore, notice pane C (CCWMA3): plants in the elevated substrate pH were less stunted and less chlorotic.

Figure 3.4 Expt. 2: (A) ‘Cully’ in the elevated substrate treatment; notice severely chlorotic leaves. (B) ‘BNMTF’ in the elevated pH substrate (pH = 7.00); showing typical interveinal chlorosis. (C) four representative replications of CCWMA3 in the elevated pH substrate treatment where the uppermost foliage is much less chlorotic than ‘Cully’ in pane (C) and ‘BNMTF’.

Table 3.1 Seed source materials information used for Expt. 1 including inventory number (Inv. No.) and suffixes (Inv. Suffix), locations, and soil physical and chemical properties for each location of open- pollinated of *Betula nigra* L. in Iowa collected by NCRPIS ARS - USDA, Ames, IA in June 2014.

Source Designation	Inv. No.	Inv. Suffix	Site Name and Latitude, Longitude	Soil Surface Texture [z]	Soil depth (cm)	Soil pH [z]	P [z] ($mg \cdot kg^{-1}$)	K [z] ($mg \cdot kg^{-1}$)	Ca [z] ($mg \cdot kg^{-1}$)	Mg [z] ($mg \cdot kg^{-1}$)	Zn [z] ($mg \cdot kg^{-1}$)	Fe [z] ($mg \cdot kg^{-1}$)
BSP1	32386	14ncao08	Bearbower Sand Prairie - Buchanan Co., IA	Loam	0 - 15.24	6.90	21.0	220	2484	269	5.5	110
			42.304102, -91.928265		15.25 - 30.48	6.40	14.0	193	1713	267	0.20	106
BSP2	32386	14ncao04	Bearbower Sand Prairie - Buchanan Co., IA	Loamy fine sand	0 - 15.24	5.00	23.0	48.0	823.0	83.0	2.0	382
			42.301119, -91.928144		15.25 - 30.48	4.80	65.0	26.0	566.0	52.0	0.40	252
BSP3	32386	14ncao03	Bearbower Sand Prairie - Buchanan Co., IA	Loamy fine sand	0 - 15.24	5.15	11.0	32.0	452.0	52.0	0.80	156
			42.300178, -91.926969		15.25 - 30.48	5.00	10.0	14.0	153.0	24.0	0.30	155
CF1	32387	14ncao03	Ciha Fen - Johnson Co., IA	Loamy fine sand	0 - 15.24	5.05	23.0	41.0	501.0	87.0	0.60	135
			41.830399, -91.382456		15.25 - 30.48	5.00	16.0	21.0	173.0	45.0	0.00	102
CF2	32387	14ncao04	Ciha Fen - Johnson Co., IA	Loamy fine sand	0 - 15.24	6.60	23.0	41.0	501.0	87.0	0.60	138
			41.830592, -91.381484		15.25 - 30.48	6.20	16.0	21.0	173.0	45.0	0.00	125
PWMA1	32388	14ncao03	Princeton WMA - Scott Co., IA	Loamy fine sand	0 - 15.24	7.10	41.0	114	2734	396	5.9	296
			41.724055, -90.348031		15.25 - 30.48	7.15	26.0	98.0	2213	338	4.10	223
PWMA2	32388	14ncao04	Princeton WMA - Scott Co., IA	Clay loam	0 - 15.24	6.05	34.0	118	2574	423	4.5	547
			41.724464, -90.346159		15.25 - 30.48	5.80	27.0	56.0	1300	256	1.6	556
PWMA3	32388	14ncao02	Princeton WMA - Scott Co., IA	Slit loam	0 - 15.24	5.80	32.0	142	3182	868	2.0	x
			41.693487, -90.340461		15.25 - 30.48	3.60	25.0	127	3108	890	1.7	x
CCWMA1	32389	14ncao01	Clemons Creek WMA - Washington Co.	Clay loam	0 - 15.24	7.25	63.0	119	2376	383	2.6	368
			41.303883, -91.743425		15.25 - 30.48	7.15	60.0	90.0	2358	421	2.4	362
CCWMA2	32389	14ncao02	Clemons Creek WMA - Washington Co.	Silt loam	0 - 15.24	7.00	72.0	111	2563	438	2.7	436
			41.303358, -91.738531		15.25 - 30.48	7.10	75.0	100	2858	478	3.0	420

[z] Soil physical and chemical property tests were conducted by the Soil and Plant Analysis lab at Iowa State University, Ames IA. Soil pH and nutrients P, K, Ca, Mg, Zn, and Fe determined using the Mehlich-3 Extractant method.

Table 3.2 Seed source materials information used for Expt. 2 including inventory number (Inv. No.) and suffixes (Inv. Suffix), locations, and soil physical and chemical properties for each location of open- pollinated of *Betula nigra* L. in Iowa collected by NCRPIS ARS - USDA, Ames, IA in June 2014.

Source Designation	Inv. No.	Inv. Suffix	Site Name and Latitude, Longitude	Surface Texture [z]	Soil depth (cm)	Actual pH [z]	P [z] (mg·kg^{-1})	K [z] (mg·kg^{-1})	Ca [z] (mg·kg^{-1})	Mg [z] (mg·kg^{-1})	Zn [z] (mg·kg^{-1})	Fe [z] (mg·kg^{-1})
CCWMA2	32389	14ncao02	Clemons Creek WMA - Washington Co.	Silt loam	0 - 15.24	7.00	72.0	111	2563	438	2.7	436
			41.303358, -91.738531		15.25 - 30.48	7.10	75.0	100	2858	478	3.0	420
CCWMA3	32389	14ncao03	Clemons Creek WMA - Washington Co.	Silt loam	0 - 15.24	7.05	92.0	123	2964	574	4.5	389
			41.302854, -91.738201		15.25 - 30.48	6.85	66.0	99.0	2800	616	3.7	360
CF3	32387	14ncao05	Ciha Fen - Johnson Co., IA	Loamy fine	0 - 15.24	7.45	41.0	68.0	1272	185	1.0	96.0
			41.846586, -91.384452	sand	15.25 - 30.48	7.40	38.0	41.0	775.0	159	0.7	108
PWMA2	32388	14ncao04	Princeton WMA - Scott Co., IA	Clay Loam	0 - 15.24	6.05	34.0	118	2574	423	4.5	547
			41.724464, -90.346159		15.25 - 30.48	5.80	27.0	56.0	1300	256	1.6	556

[z] Soil physical and chemical properties tests were conducted by the Soil and Plant Analysis lab at Iowa State University, Ames IA. Soil pH and nutrients P, K, Ca, Mg, Zn, and Fe determined using the Mehlich-3 Extractant method.

Table 3.3 Number of replications (*n*) of *Betula nigra* sources used per treatment used for Expt. 2.

Source	***n***	
	Control pH Substrate (pH = 5.29)	Elevated pH Substrate (pH = 7.00)
'BNMTF'	6	6
OP 'BNMTF'	8	8
CCWMA2	8	8
CCWMA3	8	7
CF3	5	5
'Cully'	7	8
PWMA2	5	7

Table 3.4 Expt. 1: *P* values for main effects and interactions assessed by analysis of variance for growth characteristics and SPAD, chlorophylls a and b, and micro- and macronutrients of leaves after 121 d from Iowa seed sources of *Betula nigra* collected by the NCRPIS ARS - USDA, Ames, IA subjected to 120 mL drenches, twice- weekly, of either 0% (control) or 4.77% flowable $CaCO_3$ (elevated).

Significance (*P* value)	**Plant height**	**Stem diameter**	**Number of leaves**	**Total leaf area**	**Leaf dry weight**	**Shoot dry weight**
Substrate pH [z]	<0.0001[x]	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Seed Source [y]	<0.0001	<0.0001	<0.0001	<0.0001	0.0002	<0.0001
Seed Source x Substrate pH	0.0195	0.0088	0.0363	0.1843	0.0128	0.0286

	SPAD (119 DAP)	**leaf chlorophyll a and b**	**Fe**	**Mn**	**Cu**	**Zn**
Substrate pH	<0.0001	0.0108	<0.0001	<0.0001	<0.0001	<0.0001
Seed Source	0.0098	<0.0001	0.5421	0.0137	0.1776	0.0005
Seed Source x Substrate pH	0.0453	0.1139	0.8126	0.0453	0.0729	0.0255

	N	**P**	**P:Fe**	**K**	**Ca**	**Mg**
Substrate pH	0.1691	0.2170	<0.0001	<0.0001	<0.0001	<0.0001
Seed Source	0.0035	0.0293	0.0071	0.0144	0.0961	0.0571
Seed Source x Substrate pH	0.0664	0.0186	0.0248	0.3565	0.1871	0.1059

[z] Two substrate pH treatments: 120 mL 0% (control) and 4.77% flowable $CaCO_3$ (elevated)

[y] Clones of progeny from Iowa seed sources of *B. nigra* collected by the NCRPIS ARS - USDA, Ames, IA, industry cultivars, and an open- pollinated 'BNMTF'

[x] Analysis of variance carried out using proc GLM. When main effects and interactions were significant *P* values and $_{LSM}$ differences were assessed at $P \leq 0.05$ with Fisher's Protected LSD in SAS® University Edition (2018).

Table 3.5 Expt. 1: Effects of substrate pH and seed source on plant heights, stem diameters, total number of leaves, and total leaf and shoot dry weights of *Betula. nigra* seed sources.

Seed Source [z]	Treatment [y]	Plant height (cm)	Stem diameter (mm)	Number of leaves (count)	Leaf dry weight (g)	Shoot dry weight (g)
OP 'BNMTF'	Control	95.45 ± 6.4 bcdef [x]	11.0 ± 0.264 cde	188.3 ± 21.6 a	8.46 ± 0.77 abcde	13.2 ± 1.2 abc
	Elevated	101.7 ± 4.5 abcd	9.66 ± 0.432 fgh	111.8 ± 6.28 defg	6.36 ± 0.39 fg	9.00 ± 1.12 ef
BSP1	Control	101.8 ± 4.3 abcd	9.86 ± 0.418 efgh	113 ± 14.1 defg	8.09 ± 0.92 bcdef	12.5 ± 0.91 bc
	Elevated	70.99 ± 4.1 hi	8.08 ± 0.394 k	71.8 ± 13.1 hi	3.89 ± 0.61 hi	6.23 ± 0.63 g
BSP2	Control	81.41 ± 8.1 fgh	9.16 ± 0.534 hij	138.0 ± 16.3 cdef	8.19 ± 0.50 bcdef	9.27 ± 1.0 ef
	Elevated	59.41 ± 11 i	8.58 ± 0.417 ijk	66.30 ± 13.7 i	3.37 ± 0.53 i	5.93 ± 0.59 g
BSP3	Control	115.3 ± 5.1a	10.6 ± 0.401 fghi	137.1 ± 9.34 cdef	7.03 ± 1.0 defg	12.4 ± 1.5 cde
	Elevated	93.68 ± 5.6 bcdefg	9.55 ± 0.281 ijk	96.50 ± 7.42 ghi	5.94 ± 0.49 g	9.43 ± 0.63 ef
CCWMA1	Control	104.86 ± 4.8ab	11.7 ± 0.406 ab	158.8 ± 12.2 bcd	9.78 ± 0.39 ab	15.2 ± 1.1 a
	Elevated	84.49 ± 4 efgh	10.3 ± 0.231 cdefg	140.3 ± 13.1 abc	6.91 ± 0.67 efg	10.9 ± 0.84 cde
CCWMA2	Control	88.13 ± 5.8 cdefg	11.2 ± 0.380 abc	126.6 ± 10.5 cdefg	8.44 ± 0.75 abcde	11.2 ± 1.1 cde
	Elevated	78.16 ± 5.6 gh	9.90 ± 0.277 efgh	111.1 ± 16.3 defgh	5.77 ± 0.63 gh	8.2 ± 0.94 fg
CF1	Control	102.3 ± 3.3 abc	11.2 ± 0.509 abc	134.5 ± 6.16 cdefg	7.86 ± 0.79 cdef	12.7 ± 0.90 abc
	Elevated	97.22 ± 4.0 bcdef	9.34 ± 0.353 ghi	102.9 ± 9.46 efghi	7.16 ± 0.65 cdefg	10.7 ± 0.74 cdef
CF2	Control	107.6 ± 4.5 ab	12.1 ± 0.364 a	144.4 ± 12.8 cde	10.2 ± 0.70 a	14.9 ± 0.71 ab
	Elevated	85.36 ± 7.0 defgh	10.0 ± 0.403 defgh	104.4 ± 15.3 efghi	5.65 ± 0.84 gh	9.61 ± 0.99 ef
PWMA1	Control	87.65 ± 5.1 bcdefg	12.1 ± 0.311 a	182.9 ± 29.1 ab	6.87 ± 0.78 efg	11.1 ± 0.41 cde
	Elevated	93.68 ± 5.6 cdefg	10.2 ± 0.318 cdefgh	100.5 ± 11.4 fghi	5.67 ± 0.44 gh	11.0 ± 1.0 cde
PWMA2	Control	102.7 ± 3.8 abc	11.2 ± 0.184 abc	148.8 ± 9.52 bcd	8.89 ± 0.85 abcd	14.2 ± 0.72 ab
	Elevated	98.07 ± 6.1 bcdef	10.8 ± 0.451 bcde	156.5 ± 19.4 bcd	7.95 ± 0.40 bcdef	13.9 ± 0.66 ab
PWMA3	Control	99.00 ± 8.9 abcde	11.7 ± 0.287 ab	123.8 ± 10.3 cdefg	8.99 ± 0.63 abc	14.1 ± 1.2 ab
	Elevated	97.14 ± 7.5 bcdef	8.15 ± 0.396 jk	104.9 ± 11.6 efghi	6.46 ± 0.83 fg	9.8 ± 1.1 def

[z] Iowa *B. nigra* collected by the NCRPIS ARS - USDA, Ames, IA and OP 'BNMTF' collected from a residential tree

[y] two substrate pH treatments: 120 mL 0% (control) and 4.77% flowable $CaCO_3$ (elevated). Control (pH = 5.76) and elevated (pH = 7.57).

[x] Treatments means ± 1 SE of growth parameters after 121 d. Values in the same column sharing the same letter do not differ statistically. *P* values and $_{LSM}$ differences were assessed at $P \leq 0.05$ using Proc GLM/lsmeans option with Fisher's Protected LSD in SAS® University Edition (2018).

Table 3.6 Expt. 1: Effects of substrate pH and seed source on leaf chlorophyll a and b, and micronutrient concentrations from *Betula nigra* seed sources.

Factor	leaf chlorophyll a and b ($\mu g \cdot cm^{-2}$)	Fe ($mg \cdot kg^{-1}$)	Mn ($mg \cdot kg^{-1}$)	Mn:Fe	Cu ($mg \cdot kg^{-1}$)	Zn ($mg \cdot kg^{-1}$)
Seed Source [z]						
OP 'BNMTF'	18.62 c [x]	51.83	354.0 c	5:1 bc	4.51	129.0 bcd
BSP1	20.77 bc	53.17	390.2 bc	7:1 bc	8.15	141.4 bcd
BSP2	21.65 bc	51.75	756.2 a	14:1 a	4.16	197.2 a
BSP3	25.86 a	52.65	471.2 bc	7:1 bc	4.39	147.4 bcd
CCWMA1	23.90 ab	51.59	409.1 bc	7:1 bc	4.29	118.0 d
CCWMA2	22.76 ab	61.86	420.6 bc	6:1 bc	4.24	152.0 bc
CF1	21.78 bc	48.99	316.5 c	5:1 bc	4.00	132.0 bcd
CF2	21.48 bc	47.61	596.3 ab	10:1 ab	3.74	160.2 b
PWMA1	18.79 c	39.38	547.8 abc	11:1 ab	4.48	151.6 bc
PWMA2	20.75 bc	50.64	319.6 c	5:1 c	3.98	125.6 bc
PWMA3	21.99 bc	41.23	402.0 bc	8:1 bc	4.60	129.3 bcd
Substrate pH [y]						
Control	26.39 a	72.10 a	842 b	13:1 a	6.61 a	185.8 a
Elevated	16.95 b	28.02 b	63.6 a	2:1 b	2.57 b	102.3 b

[z] Iowa *B. nigra* collected by the NCRPIS ARS - USDA, Ames, IA and OP 'BNMTF' collected from a residential tree

[y] Two substrate pH treatments 120 mL 0 (control) and 4.77% flowable $CaCO_3$ (elevated). Control (pH = 5.76) and elevated (p = 7.57)

[x] The source means ± 1 SE for leaf chlorophyll a and b and micronutrient concentrations after 121 d. The treatment column refers to the mean the substrate leachate pH values for treatment groups. Columns sharing the same letter do not differ statistically. *P* values for source and substrate pH comparisons were assessed at $P \leq 0.05$ using Proc GLM/lsmeans option with Fisher's Protected LSD in SAS® University Edition (2018).

Table 3.7 Expt. 1: Effects of substrate pH and seed source on leaf SPAD after 119 d and micronutrient analysis from leaves from *Beutla nigra* seed sources.

Seed Source [z]	Treatment [y]	SPAD (119 DAP)	P %	P:Fe (%:%)	Mn (mg·kg^{-1})	Zn (mg·kg^{-1})
OP 'BNMTF'	Control	33.79 ± 0.56 a [x]	0.241 ± 0.021 ab	32:1 fg	662.5 ± 83.8 d	167.1 ± 10.9 bcde
	Elevated	18.19 ± 2.9 ef	0.258 ± 0.013 a	91:1 ab	45.5 ± 2.67 e	92.7 ± 2.6 gh
BSP1	Control	35.30 ± 0.75 a	0.183 ± 0.01 cd	27:1 fg	720 ± 165 cd	175.4 ± 24.0 bcde
	Elevated	20.02 ± 3.3 def	0.263 ± 0.02 a	94 :1 ab	60.37 ± 11.4 e	107.4 ± 3.61 gh
BSP2	Control	34.05 ± 0.66 a	0.243 ± 0.0070 ab	44 :1 ef	1423 ± 382 a	285.7 ± 33.6 a
	Elevated	21.42 ± 3.4 cde	0.200 ± 0.019 bcd	62:1 cde	88.97 ± 10.57 e	108.6 ± 7.48 gh
BSP3	Control	32.98 ± 0.54 a	0.257 ± 0.0302 a	33:1 fg	885.7 ± 167 bcd	194.7 ± 16 bcd
	Elevated	27.29 ± 2.9 bc	0.185 ± 0.015 cd	74:1 bcd	56.73 ± 6.25 e	100.0 ± 8.64 gh
CCWMA1	Control	33.54 ± 0.67 a	0.205 ± 0.022 bcd	29:1 fg	764.1 ± 124 cd	153.0 ± 20.1 edf
	Elevated	27.09 ± 3.1 bc	0.202 ± 0.010 bcd	66:1 cd	54.00 ± 4.34 e	83.10 ± 7.03 h
CCWMA2	Control	35.98 ± 0.88 a	0.224 ± 0.024 abc	25:1 fg	763.8 ± 73.6 cd	197.9 ± 24.6 bc
	Elevated	23.43 ± 2.1 cde	0.166 ± 0.0050 d	58:1 de	77.68 ± 9.61 e	106.1 ± 2.02 gh
CF1	Control	32.01 ± 0.47 ab	0.233 ± 0.018abc	37:1 fg	576.9 ± 53.6 d	167.3 ± 8.79 bcde
	Elevated	23.38 ± 2.7 cde	0.22 ± 0.010 bc	72:1 cd	56.13 ± 9.48 e	96.60± 10.37 gh
CF2	Control	35.09 ± 0.97 a	0.187 ± 0.015 cd	29:1 fg	1115 ± 117 ab	191.6 ± 23.7 bcd
	Elevated	22.80 ± 3.0 cde	0.187 ± 0.012 cd	66:1 cd	76.83 ± 14.4 e	128.9 ± 26.2 fgh
PWMA1	Control	32.38 ± 0.68 ab	0.225 ± 0.023 abc	38:1 fg	1007 ± 114 bc	199.7 ± 14.7 b
	Elevated	14.39 ± 2.3 f	0.210 ± 0.025 abcd	105:1 a	88.07 ± 3.83 e	103.6 ± 7.00 gh
PWMA2	Control	34.09 ± 0.93 a	0.223 ± 0.020 abc	31:1 fg	596.8 ± 9.52 d	154.9 ± 4.11 ed
	Elevated	21.44 ± 2.4 cde	0.242 ± 0.015 ab	82:1 bc	42.35 ± 1.38 e	96.30 ± 5.59 gh
PWMA3	Control	35.78 ± 0.70 a	0.203 ± 0.016 bcd	36:1 fg	751.2 ± 42.9 cd	156.6 ± 7.26 cde
	Elevated	25.67 ± 3.0 cd	0.185 ± 0.019 cd	80:1 bc	52.87 ± 6.61 e	102.0 ± 8.21 gh

[z] Iowa *B. nigra* collected by the NCRPIS ARS - USDA, Ames, IA and OP 'BNMTF' collected from a residential tree.

[y] Two substrate pH treatments 120 mL 0% (control) and 4.77% flowable $CaCO_3$ (elevated). Control (pH = 5.76) and elevated (pH = 7.57).

[x] Treatment means ± 1SE of leaf SPAD and micronutrient concentrations. The treatment column refers to the mean the substrate leachate pH values for Values in the same column sharing the same letter do not differ statistically. *P* values and $_{LSM}$ differences were assessed at $P \leq 0.05$ using Proc GLM/lsmeans option with Fisher's Protected LSD in SAS® University Edition (2018).

Table 3.8 Expt. 1: Effects of substrate pH and seed source on leaf macronutrient analysis from leaves of *Betula nigra* seed sources.

Factor	N %	P %	K %	Ca %	Mg %
Seed Source [z]					
OP 'BNMTF'	2.77 a [x]	0.250 a	1.64 a	0.903	0.427
BSP1	2.36 bcd	0.223 abcd	1.33 bc	0.952	0.399
BSP2	2.29 bcd	0.221 abcd	1.45 ab	0.939	0.384
BSP3	2.49 bc	0.221 abcd	1.26 bc	0.923	0.397
CCWMA1	2.39 bcd	0.203 bcd	1.16 c	0.886	0.381
CCWMA2	2.24 cd	0.195 cd	1.44 ab	1.04	0.319
CF1	2.42 bcd	0.227 abc	1.46 ab	0.838	0.407
CF2	2.16 d	0.187 d	1.39 bc	0.977	0.38
PWMA1	2.37 bcd	0.217 abcd	1.44 ab	0.830	0.378
PWMA2	2.55 ab	0.232 ab	1.36 bc	0.845	0.357
PWMA3	2.45 bc	0.194 cd	1.23 bc	0.862	0.341
Substrate pH [y]					
Control	2.45	0.220	1.13 b	0.708	0.333 b
Elevated	2.37	0.211	1.63 a	1.11	0.443 a

[z] Iowa *B. nigra* collected by the NCRPIS ARS - USDA, Ames, IA and OP 'BNMTF' collected from a residential tree

[y] Two substrate pH treatments: 120 mL 0% (control) and 4.77% flowable $CaCO_3$ (elevated)

[x] Seed source means ± 1SE for macro- nutrient concentrations taken from leaves after 121 d. Values in the same column sharing the same letter do not differ statistically. *P* values for seed source and substrate pH factors were assessed at $P \leq 0.05$ using Proc GLM/lsmeans option with Fisher's Protected LSD in SAS® University Edition (2018).

Table 3.9 Expt. 2: *P* values for main effects and interactions for growth characteristics and SPAD, chlorophylls a and b, and micro- and macronutrients of leaves from clones of progeny from Iowa seed sources of *Betula nigra* collected by the NCRPIS ARS - USDA, Ames, IA, industry cultivars, and an open- pollinated 'BNMTF'.

Significance (*P* value)	**Mean stem length**	**Mean stem diameter**	**Number of leaves**	**Total leaf area**	**Leaf dry weight**	**Shoot dry weight**
Substrate pH [z]	0.0002 [x]	0.0012	<0.0001	<0.0001	<0.0001	<0.0001
Source [y]	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0003
Source x Substrate pH	0.2987	0.6214	0.9175	0.0825	0.0635	0.1565

	SPAD	**leaf chlorophyll**	**Fe**	**Mn**	**Cu**	**Zn**
Substrate pH	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Source	<0.0001	0.0001	<0.0001	<0.0001	0.3838	<0.0001
Source x Substrate pH	0.2350	0.1565	0.0228	<0.0001	0.6651	0.0002

	N	**P**	**P:Fe**	**K**	**Ca**	**Mg**
Substrate pH	0.0031	0.0437	<0.0001	<0.0001	<0.0001	<0.0001
Source	0.0010	<0.0001	0.0006	0.4495	0.0003	0.0138
Source x Substrate pH	0.0900	0.0011	0.0003	0.4120	0.0883	0.8692

[z] Two substrate pH treatments: 120 mL 0% (control) and 4.77% flowable $CaCO_3$ (elevated)

[y] Clones of progeny from Iowa seed sources of *B. nigra* collected by the NCRPIS ARS - USDA, Ames, IA, industry cultivars, and an open- pollinated 'BNMTF'

[x] Analysis of variance carried out using proc GLM. When main effects and interactions were significant *P* values and $_{LSM}$ differences were assessed at $P \leq 0.05$ with Fisher's Protected LSD in SAS® University Edition (2018).

Table 3.10 Expt. 2: Effects of source and substrate pH on growth characteristics, leaf SPAD ratings, and leaf chlorophylls a and b after 103 d from clones of progeny from Iowa seed sources of *Betula nigra* collected by the NCRPIS ARS - USDA, Ames, IA, industry cultivars, and an open- pollinated 'BNMTF'.

Factor	Mean stem length (cm)	Mean stem diameter (mm)	Number of leaves (count)	Total leaf area (cm^2)	Leaf dry weight (g)	Shoot dry weight (g)	SPAD	leaf chlorophyll a and b ($\mu g \cdot cm^{-2}$)
Source [z]								
'BNMTF'	35.57 bc [x]	2.887 cd	119.3 ab	1167.0 c	6.765 b	4.462 ab	32.08 ab	25.70 ab
OP 'BNMTF'	31.38 c	2.667 d	132.6 a	1561.8 b	7.720 b	5.456 a	26.88 c	21.01 d
CCWMA2	46.41 a	3.742 a	86.25 c	2144.8 a	11.41 a	5.638 a	31.29 b	24.01 bc
CCWMA3	32.71 bc	3.070 cd	74.72 c	1305.6 bc	7.129 b	3.240 b	35.46 a	26.78 a
CF3	34.35 bc	3.231 bc	68.10 c	1240.1 bc	5.731 b	2.938 b	30.78 b	24.95 abc
'Cully'	29.27 c	2.958 cd	86.60 c	1347.0 bc	6.364 b	3.433 b	26.51 c	21.87 cd
PWMA2	39.66 ab	3.700 ab	94.60 bc	1618.9 b	7.922 b	5.020 a	30.89 b	24.13 abc
Substrate pH [y]								
Control	39.59 a	3.401 a	113 a	1891.9 a	6.45 a	5.82 a	36.44 a	28.73 a
Elevated	31.66 b	2.970 b	76.0 b	1075.3 b	5.71 b	2.81 b	24.67 b	19.40 b

[z] *B. nigra* clones from progeny of provenances collected by the NCRPIS ARS - USDA, Ames, IA, trade standards 'BNMTF' and 'Cully', and OP 'BNMTF' seed source from a residential tree

[y] Two substrate pH treatments: 120 mL 0 (control) and 4.77% flowable $CaCO_3$ (elevated). Control (pH = 5.29) and elevated (pH = 7.00).

[x] Soure means ± SE of growth parameters. Columns sharing the same letter do not differ statistically. *P* values for seed source and substrate pH factors were assessed at $P \leq 0.05$ using Proc GLM/lsmeans option with Fisher's Protected LSD in SAS® University Edition (2018).

Table 3.11 Expt. 2: Effects of source and substrate pH on total leaf N, K, Ca, Mg, Cu after 103 d from clones of progeny from Iowa seed sources of *Betula nigra* collected by the NCRPIS ARS - USDA, Ames, IA, industry cultivars, and an open-pollinated 'BNMTF'.

Factor	N %	K %	Ca %	Mg %	Cu ($mg \cdot kg^{-1}$)
Source [z]					
'BNMTF'	3.60 ab [x]	1.93	1.21 bcd	0.341 a	2.57
OP 'BNMTF'	3.19 c	2.02	1.03 d	0.262 bc	1.8
CCWMA2	3.36 bc	1.97	1.19 cd	0.256 c	3.15
CCWMA3	3.18 c	2.02	1.13 cd	0.262 bc	2.14
CF3	3.08 c	2.13	1.32 abc	0.335 ab	2.83
'Cully'	3.65 a	2.05	1.50 a	0.342 a	1.94
PWMA2	3.32 bc	2.39	1.40 ab	0.332 ab	2.66
Substrate pH [y]					
Control	3.46 a	1.64 b	1.08 b	0.264 b	4.02 a
Elevated	3.22 b	2.50 a	1.43 a	0.345 a	0.854 b

[z] *B. nigra* clones from progeny of provenances collected by the NCRPIS ARS - USDA, Ames, IA, trade standards 'BNMTF' and 'Cully', and OP 'BNMTF' seed source from a residential tree

[y] two substrate pH treatments: 120 mL 0 (control) and 4.77% flowable $CaCO_3$ (elevated). Control (pH = 5.29) and elevated (pH = 7.00)

[x] Source means ± 1SE. Columns sharing the same letter do not differ statistically. *P* values for source and substrate pH were assessed at $P \leq 0.05$ using Proc GLM/lsmeans option with Fisher's Protected LSD in SAS® University Edition (2018).

Table 3.12 Expt. 2: Effects of substrate pH and seed source on leaf micronutrient analysis after 103 d from clones of progeny from Iowa seed sources of *Betula nigra* collected by the NCRPIS ARS - USDA, Ames, IA, industry cultivars, and an open- pollinated 'BNMTF'.

Seed Source [z]	Treatment [y]	P %	Fe (mg·kg^{-1})	P:Fe (%:%)	Mn (mg·kg^{-1})	Mn:Fe	Zn (mg·kg^{-1})
'BNMTF'	Control	0.324 ± 0.0032 cde [x]	49.43 ± 3.7 ab	1:1 d	3721 ± 182 b	75:1 b	347 ± 11 bc
	Elevated	0.361 ± 0.0029 c	19.17 ± 0.46 ghi	10:1 c	363.0 ± 17.7 f	19:1 de	144 ± 12 ef
OP 'BNMTF'	Control	0.270 ± 0.014 ef	33.4 ± 2.0 de	1:1 d	2604 ± 232 de	78:1 b	251 ± 30 d
	Elevated	0.327 ± 0.020 cd	18.3 ± 1.7 hi	14:1 b	235.7 ± 23.3 f	13:1 ef	120 ± 18 f
CCWMA2	Control	0.349 ± 0.013 cd	42.95 ± 1.7 bc	2:1 d	2226 ± 120 e	52:1 c	307 ± 12 ef
	Elevated	0.307 ± 0.012 de	14.63 ± 0.76 i	8:1 c	365.8 ± 18.9 f	25:1 d	161 ± 5.8 ef
CCWMA3	Control	0.353 ± 0.019 cd	44.18 ± 1.3 bc	1:1 d	3172 ± 170 c	72:1 b	366 ± 23 b
	Elevated	0.246 ± 0.012 f	27.13 ± 1.8 efg	8:1 c	334.7 ± 61.0 f	12:1 ef	161 ± 10 ef
CF3	Control	0.329 ± 0.019 cd	55.65 ± 2.0 a	1:1 d	3212 ± 35.6 bc	58:1 c	350 ± 7.1 bc
	Elevated	0.325 ± 0.0050 cde	39.6 ± 5.7 cd	16:1 ab	207.5 ± 31.7 f	5:1 f	140 ± 1.5 ef
'Cully'	Control	0.536 ± 0.020 a	52.53 ± 3.6 a	1:1 d	5059 ± 467 a	95:1 a	478 ± 20 a
	Elevated	0.478 ± 0.036 b	22.98 ± 3.6 fgh	18:1 a	266.0 ± 12.3 f	12:1 ef	167 ± 18 e
PWMA2	Control	0.355 ± 0.021 cd	56.15 ± 8.0 a	1:1 d	2952 ± 506 cd	52:1 c	326 ± 4.9 bc
	Elevated	0.317 ± 0.0078 cde	29.03 ± 5.0 ef	11:1 c	304.0 ± 34.1 f	11:1 ef	162 ± 1.5 ef

[z] two substrate pH treatments: 120 mL 0 (control) and 4.77% flowable $CaCO_3$ (elevated). Control (pH = 5.29) and elevated (pH = 7.00).

[y] *B. nigra* clones from progeny of provenances collected by the NCRPIS ARS - USDA, Ames, IA, trade standards 'BNMTF' and 'Cully', and OP 'BNMTF' seed source from a residential tree

[x] Treatment means ± SE leaf SPAD and micronutrient concentrations. Columns sharing the same letter do not differ statistically. *P* values and $_{LSM}$ differences were assessed at $P \leq 0.05$ using Proc GLM/lsmeans option with Fisher's Protected LSD in SAS® University Edition (2018).

Appendix A - SPAD Ratings from Leaves of Durand, Lacey, and Pin Oak in Elevated pH Substrate

Abstract

We attempted to evaluate the iron (Fe)-induced interveinal foliar chlorosis (IFC) of eight collections of oaks (*Quercus* L.) comprised of seven species in elevated pH substrate: sawtooth oak (*Quercus acutissima* Carruthers), chestnut oak (*Quercus montana* Willd.), chinquapin oak (*Quercus muehlenbergii* Engelm.), bur oak (*Q. macrocarpa* Michx.), Lacey oak (*Q. laceyi* Small), pin oak (*Q. palustris* L.), and two collections of Durand oak [*Q. sinuata* var. *breviloba* (Torr.) C.H. Mull.]. Due to factors such as poor substrate quality, seed size differences, salt stress, and differences in growth, leaf SPAD values were only collected from *Q. laceyi*, one collection of *Q. sinuata* var. *breviloba* (Lost Maples State Natural Area), and *Q. palustris* KSU after 265 d. We used the SPAD values as an indicator to their tolerance to IFC in an elevated pH substrate.

Introduction to Lacey Oak

Quercus laceyi was first discovered by the English naturalist, Howard Lacey in the late 1800's on his ranch in Kerr Co., Texas (Petrides and Petrides 1992). Unlike assigning incorrect names for Texas red oak (*Quercus buckleyi* Nixon & Dorr) and *Q. sinuata* var. *breviloba* the first time they were described, the correct distinction as a new species was given to Lacey oak (*Quercus laceyi*) in honor of Howard Lacey (Small, 1901). Other taxonomists reclassified the species as *Quercus brevifolia* now *Q. sinuata* var. *breviloba* and then, considered it was a synonym of *Quercus glaucoides* Mart. Gal. an Mexican evergreen species discovered in 1843) (Muller, 1970; Trelease, 1924). Nixon and Muller (1992) explain that *Q. laceyi* and *Q.*

glaucoides are two distinct species, because *Q. glaucoides* has fused cotyledons and is evergreen; *Q. laceyi* has neither of these traits. Furthermore, *Q. laceyi* has thinner leaves, more attenuated lobes, deeper sinuses, and sessile to sub-sessile fruit compared to *Q. glaucoides* (Nixon, 1985; Nixon and Muller, 1992). Some plant nurseries and researchers continue to call *Q. laceyi Q. glaucoides* as it is still a synonym (Nixon and Muller, 1992).

Q. laceyi is 12 to twelve southcentral Texas counties (USDA hardiness zones 9a - 8a) and northern regions of Mexico (Stein et al., 2003). The closely related relatives for Edwards Plateau *Q. laceyi* is still a topic of discussion, because their distribution is not harmonious with the distribution of eastern U.S. complex of species of *Quercus* in the section *Quercus* (white oaks) even though *Q. laceyi* shares leaf morphological traits with the white oak (Nixon and Muller, 1992).

Q. laceyi is a small to medium sized tree potentially growing to 18.3 m in height and about as wide (Stein et al., 2003). Again, this species is not common in landscapes throughout the Midwest most likely due to it not being cold hardy enough in lower zones. Unlike many species of *Quercus*, *Q. laceyi* is probably one of the most ornamental oaks in the summer, because it showcases its pinky-peach developing foliage, maturing into blueish-green leaves as it grows (Nixon and Muller, 1992). Similar to Texas red oak (*Q. buckleyi* Nixon and Dorr), *Q. laceyi* is an extremely drought tolerant species that may bring great potential fall color to the landscape with its burnt orange to yellow foliage (Poulos et al., 2007). In addition to being drought tolerant, *Q. laceyi* may be extremely tolerant of high soil pH since the species is normally found growing soils derived from or comprised of limestone (Stein et al., 2003).

Materials and Methods

Similar to Expt. 1 2017 (Chapter 2), an experiment was conducted to evaluate the foliar chlorosis in *Q. palustris*, *Q. sinuata* var. *breviloba*, and *Q. laceyi* when subjected to high pH substrate.

Seed Source Collection

Acorns for *Q. palustris* were collected from a mature tree on the campus of Kansas State University (KSU) (same seed source as Expt. 1 2017) that had received an Fe-sulfate injection (ferric sulfate tetrahydrate, Medi-Ject Tree Injection Systems, Lincoln, NE) by a local arborist company (Tree BioLogics Inc., Manhattan, KS) in the Spring 2016 due to a history of foliar chlorosis. Acorns of *Q. sinuata* var. *breviloba* were obtained from Lost Maples State Natural Area (SNA) Bandera Co., TX on September 18, 2016 (Latitude: 29.815871, Longitude: -99.576307); also from a single tree in a native stand. Acorns from *Q. laceyi* were collected from a mature tree approximately 10.5 m tall located on a rocky outcropping at the Lost Maples SNA Bandera Co., TX on September 18, 2016 (Latitude: 29.815264, Longitude: -99.576748).

The day after acorn collection, cupules were removed, acorns were rinsed with tap water, and float tested (Bonner and Vozzo 1987). Seeds were placed in 3.8 L polyethylene bags with eight 1.3 cm perforations to ensure adequate gas exchange. Each bag contained four sheets of moist paper towel, which were periodically re-wet to maintain high humidity. Seed were stored in the dark at 3°C until planting.

Experiment Initiation

On 8 Feb. 2017, three acorns of a species were planted into each 3.8 L container filled with a 9:1:4 (by vol.) bark (Yardcare™ Small Western Bark, Mountain West Products, Rexburg, ID.) : soil conditioner (Yardcare™ Soil PEP, Mountain West Products, Rexburg, ID.) : perlite.

Substrate was amended with 2.8 $kg \cdot m^{-3}$ Osmocote Classic® (14N-4.2P-11.6K) (Everris NA, Inc., Dublin, OH), and 0.5 $kg \cdot m^{-3}$ Micromax® (Everris NA, Inc., Dublin, OH). Substrate pH treatments were created by incorporating dolomitic lime (Deco® Lawnlime®, The Georgia Marble Co., Kennesaw, GA) at 2.4 $kg \cdot m^{-3}$ (control) or 11.9 $kg \cdot m^{-3}$ (elevated pH) four weeks after planting, all containers were thinned to one plant, leaving the most vigorous seedling.

Plants were grown on benches in a glass-house greenhouse at Kansas State University, Manhattan, KS set to 24 °C day/20 °C night for 94d and then transferred to polycarbonate greenhouse in Haysville, KS under 50% shade cloth and exposed to natural photoperiod with temperatures set at 24 °C day/20 °C night. Plants were irrigated twice weekly with 120 mL tap water for four weeks to allow the root systems to fill the containers. After four weeks of study initiation, plants were irrigated twice weekly with 120 mL solution of 0% or 4.77% liquid calcium (CalOx® pH, BioSafe Systems LLC., Hartford, CT) and 0.122% magnesium sulfate heptahydrate [($MgSO_4 \cdot 7H_2O$), PDC Brands™ Stamford, CT] in tap water to create a low and high substrate solution pH, respectively. Substrate solution pH and electrical conductivity (EC) were monitored using the pour-through technique (Wright, 1986). To remedy the high substrate solution pH, plants receiving 0% liquid calcium drench also received an acidifying drench of 60 mL tap water with 21.6% aluminum sulfate [$Al_2(SO_4)_3$; Voluntary Purchasing Groups Inc, Bonham, TX] after four weeks every four weeks to lower substrate pH. Because of a dramatic response to salt stress, plant growth for some collections was detrimentally impacted from too high of an initial aluminum sulfate application, subsequent drenches were reduced in concentration to 10.8% aluminum sulfate applied carried in 60 mL tap water. Using a SPAD meter (SPAD-502, Konica Minolta, Inc.) SPAD ratings were taken after 265 d.

Experimental Design

The experimental design was a randomized complete block design (RCBD) with a factorial arrangement of treatments (species x substrate pH). Originally, there were eight collections of comprised of seven species in combination with two substrate pH levels creating sixteen treatments. Each treatment combination was initially replicated eleven times, but some treatment replications were lost due to poor germination, salt stress from too high of aluminum sulfate. Therefore there were three seed sources with ten replications of (*Q. palustris* x control pH), and six for (*Q. sinuata var. breviloba* x elevated pH), 8 replications of (*Q. sinuata var. breviloba* x control pH), and eleven of (*Q. laceyi* x control and elevated pH) used for statistical analysis. Data were subjected to an analysis of variance using general linear model (GLM) using SAS® University Edition (SAS Institute Inc., Cary, NC). When appropriate, means were separated at $P \leq 0.05$ using Tukey's HSD.

Results

Mean pH for the control and elevated substrate treatment groups after 137 d was 4.56 and 7.37, respectively. After 265 d, *Q. palustris* KSU seedlings growing in the elevated pH substrate showed IFC and had low leaf SPAD readings (control = 37.1 and elevated = 17.6). *Q. sinuata* var. *breviloba* and *Q. laceyi* in the elevated pH substrate maintained high leaf SPAD ratings compared to their respective controls [(control = 39.1 and 40.0, respectively) and (elevated = 38.5 and 39.0, respectively)]. Leaf SPAD ratings are shown in Figure A.1.

Conclusions

Based on SPAD readings after 189 d, *Q. sinuata* var. *breviloba* and *Q. laceyi* in the elevated pH substrate did not develop symptoms of IFC and maintained similar leaf SPAD ratings compared to the controls, while, *Q. palustris* KSU in the elevated pH substrate had low

SPAD ratings and developed IFC. Further investigation into the tolerance of IFC with *Q. sinuata* var. *breviloba* and *Q. laceyi* in an elevated pH should be warranted.

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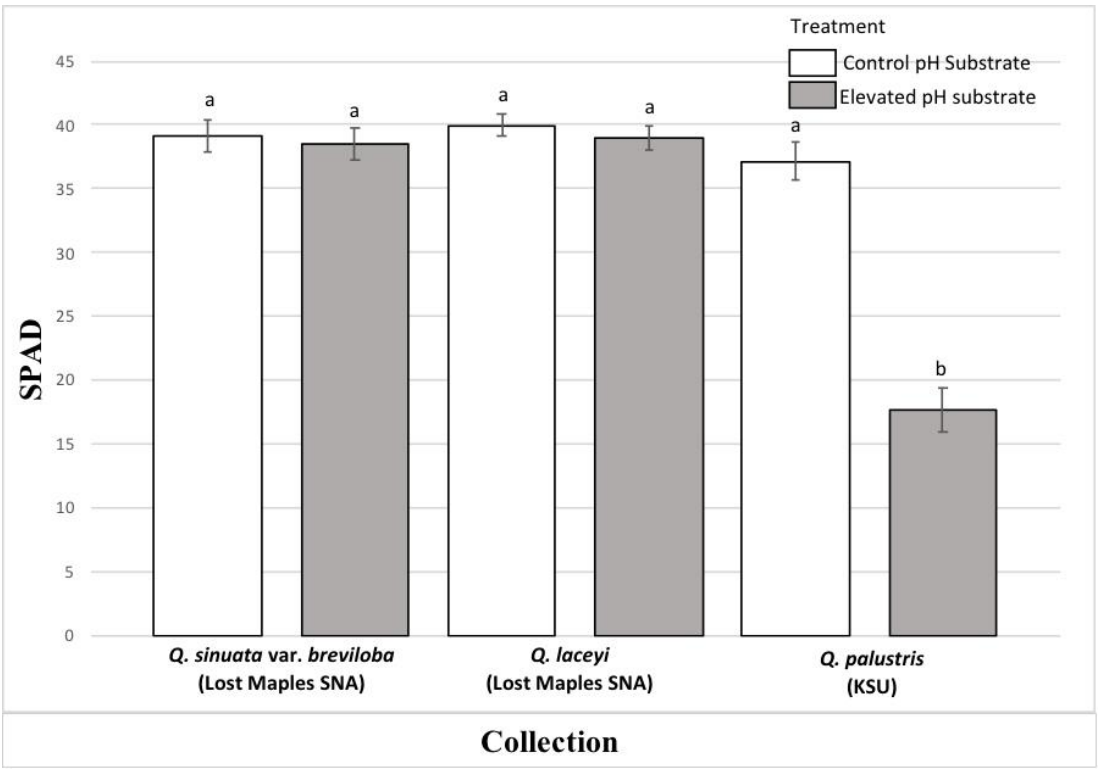

Figure A.1 SPAD ratings from leaves after 265 d for *Q. laceyi* (Lost Maples SNA), *Q. sinuata* var. *breviloba* (Lost Maples SNA), and *Q. palustris* (KSU) receiving either 120 mL of 0 (control) or 4.77% flowable $CaCO_3$ (elevated) substrate drenches twice- weekly; control (mean pH = 4.56 after 137 d) and elevated (pH = 7.37 after 137 d). Each bar represents in mean ± SE. [number of reps: (*Q. sinuata* var. *breviloba*: control = 8, elevated = 6; *Q. laceyi*: control = 11, elevated = 11; *Q. palustris*: control = 10, elevated = 11)]. Data subjected to analysis of variance using LSM differences were assessed at $P \leq 0.05$ using Proc GLM/lsmeans option with Tukey's HSD in SAS® University Edition (2018). Species * pH $P = < 0.0001$.