DETERMINING THE CAUSES OF ABIOTIC LEAF SCORCH: GETTING TO THE ROOT OF THE PROBLEM.

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A THESIS

Submitted to
Michigan State University
In partial fulfillment of the requirements
For the degree of

Plant Biology - Master of Science

2015

ABSTRACT

DETERMINING THE CAUSES OF ABIOTIC LEAF SCORCH:
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Leaf scorch is a common symptom of declining trees in urban landscapes, especially Northern red oaks, *Quercus rubra*. Established trees with a known history of abiotic leaf scorch were systematically evaluated for depth of root flare, girdling root severity, soil compaction (resistance), soil nutrient profile, pH, and soil oxygen profile. Photosynthesis efficiency, leaf pressure potential, leaf conductance, leaf transpiration, and plant tissue nutrition were measured to examine differences in physiological function between scorched and non-scorched oaks on the same site and among sites. Leaf scorch was significantly positively correlated to planting depth. Planting depth was positively correlated to girdling roots and smaller DBH. However, leaf scorch was not significantly correlated with the presence of girdling roots. Soil treatment with micro-fine sulfur, and Mauget injections increased iron but not manganese in leaf nutrient levels. Scorched trees had smaller DBH, greater planting depth than non-scorched trees. Tree height and DBH was positively correlated with less compacted soils. Scorched leaves had a significantly higher temperature compared to non-scorched. Scorched trees had lower levels of manganese, and higher levels of zinc. Phosphorus, though deficient, showed a positive correlation to leaf scorch. Photosynthetic efficiency and leaf conductance data from 2009 and 2010 were highly similar. Scholander pressure chamber data from 2008 and 2009 were highly similar. A better understanding of the functional capabilities of girdling roots in oaks is needed to better interpret some results.

For Stéphan and Josée Kurzeja

ACKNOWLEDGEMENTS

This research project was funded by the MSU GREEEN (Grant # GR08-077) to Dr. G. Adams, initiative with backing from the Michigan Nursery and Landscape Association (MNLA), for J. Paul Swartz, F. W. Telewski and G. Adams. Essential instrumentation had been funded by prior grants to F. W. Telewski. Supplemental funding was from USDA Forest Service Environmental Monitoring program on Bacterial Leaf Scorch to G. Adams. Funding was received from the W.J. Beal Botanical Garden for leaf and soil analyses. I wish to thank J. Paul Swartz and the MSU Landscape services, as well as Robert Gentry and Steve Adams of the city of Adrian, MI for contributing machinery. To Dr. Frank W. Telewski for being my major advisor, teacher, colleague and friend, thank you for everything you have done for me. Thanks also goes to Dr. Bert Cregg and Dr. Gerry Adams for being part of my committee. A very special thanks goes to Dr. Jameel "Habibi" Al-Haddad for assistance with statistical analysis. Last, but certainly not least is my wonderful and loving wife Kara, without her I could never have completed this work.

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CHAPTER 1

INTRODUCTION

Leaf scorch can have many causal agents, of which some are abiotic in nature. The causes of abiotic leaf scorch are poorly understood. However, trees exhibiting abiotic leaf scorch are found almost exclusively in developed and managed landscapes. Over the past decade there has been an increase in the scientific data that can definitively show cause and effect between nursery and landscape industry practices, decline in tree health, and tree mortality. The Nursery industry, professional landscapers and those who are involved in the growing, planting, and maintenance of trees have become more aware of the potential affect that early nursery and transplanting practices have on development of root systems and on long term tree health.

One possible root cause of abiotic leaf scorch maybe associated with a disruption of water transport from roots to leaves in an affected tree. In order to properly transport water to the leaves, trees need to have a functional root system. The challenge in diagnosing causes of root architecture problems are that the system in question may be dysfunctional, but may not be visible, rather hidden underground. If the tree was not planted at the correct depth in the nursery, and subsequent transplantings resulted in deeper transplanting at the final site, then the trees root system undergoes severe stress that can lead to long term decline.

With the invention and wide spread adoption of the Air Spade, the tree industry began to utilize this new tool as a way for professionals to begin looking at root architecture in a way not previously possible. Under the direction of Paul Swartz, Campus Arborist at Michigan State University the arboricultural staff began performing exploratory root excavations to campus trees that were showing signs of leaf scorch. These excavations were done to find possible causes for scorch and decline that had begun to show up at a variety of sites around MSU’s campus. After a number of trees had their root systems exposed, a possible pattern began to emerge. Trees that were showing signs of severe leaf scorch were also, planted too deep, and had severe root architecture problems.

To better understand the causes of leaf scorch is to better understand the many challenges facing successful management of trees in the landscape including; problems of nursery practices, planting depth, tools and equipment used for planting, root girdling, root restriction, diseases, nutritional toxicities and deficiencies, salt buildup, soil resistance and bulk density, water movement and availability. Keeping this in mind, the study of leaf scorch must be both inclusive and complex. Leaf scorch symptoms, caused by abiotic processes can be expressed as a scorched canopy leading to a slow and progressive dieback of terminal twigs, eventually leading to the mortality of entire branches and eventually tree mortality. Some trees have been observed to overcome leaf scorch. That is, the underlying reasons for scorch either no longer exist, or the tree through internal processes grows out of the condition. Mortality, dieback, and decline are most evident on trees with reoccurring severe scorch symptoms that become progressively worse year to year.

Understanding the causes of tree mortality requires a broadening of our perception of tree growth, development, time and most importantly nursery and landscape industry practices and standards. The effects of improper nursery or landscaping practices are not apparent until a tree matures or the tree experiences some environmental stress. For example, nursery production and landscape planting practices can cause girdling roots. These girdling roots do not become a problem until the trees roots and trunk grow to sufficient girth to begin constricting one another, and become strangler roots. Poor site preparation, improper planting depth or leaving the burlap sack and cordage in-tacked around a transplanted tree or any number of other factors can cause slow decline and gradually lead to mortality. Since leaf scorch is often a symptom of decline and mortality, I choose to focus on leaf scorch as a means of quantifying tree health, by looking at differing levels of leaf scorch, and comparing scorch to possible root architecture problems, planting depth, soil conditions (including: compaction, nutrient availability, oxygen content, pH, foliar nutrient content, water conductivity, photosynthetic efficiency, and leaf temperature. With these observations I proposed testing the following hypotheses:

- Excessive planting depth causes abiotic leaf scorch.
- Adverse foliar and soil nutrient content will show correlations with abiotic leaf scorch.
- Excessive planting depth induces girdling roots.
- Girdling roots cause leaf scorch.
- Soil compaction, low soil oxygen content and or adverse soil pH levels will induce abiotic leaf scorch.

In 2008 three study sites were selected on the campus of Michigan State University. The sites were selected based on observations from previous years of established *Quercus rubra*, Northern red oak, with a variety of individuals exhibiting different levels of scorch, adjacent to non-scorched oaks of the same age on similar soil types. After extensive root excavations of sites at Breslin Center (BR) and Farm Lane (FR) another trend began to appear. After our original observations it was hypothesized that planting depth and severe girdling roots were perhaps causing leaf scorch, but after excavating all the root systems at sites BR and FR we discovered that trees that had not shown signs of leaf scorch did in some cases have girdling roots and or excessive planting depths. It became clear that further test plots and more experiments were necessary to get to the root of this problem. Additional sites with similar characteristics were added from 2009 through 2012, (Table 1). Eventually, the study consisted of seven sites, five of which were of similar planting age, established and managed for roughly 20 - 30 years. Some sites received regular irrigation by subsurface sprinkler systems, while others received only natural rain fall irrigation (Table 1). The other two sites were older oaks being between 25 – 40 years old, also with a history of scorch, and were planted in similar urban conditions.

Of the original three sites established in 2008, the first site, known as (BR), which stands for, the Jack Breslin Student Events Center on the campus of Michigan State University, was split into two sites which are referred to Breslin 1, and 2, or as (BR1) and (BR2). Of the original 24 trees in the BR test site only 23 remain, as one was removed on July 28th 2009. The BR1 and BR2 sites were established based on as equal number of control and scorch trees in each plot as was possible, refer to (Figure 1).

Of the other original three sites, the Oak hill Cemetery in Adrian Michigan, referred to as (AD) and the Farm lane site on West side of Farm lane between East and West bound Shaw (Figure 2), referred to as (FR), only FR and BR continued to be used for this study due to logistic constraints and lack of travel funds. Because of this, site AD was dropped from the study after the 2009 field season. This allowed me to concentrate time and resources on the test sites at MSU. FR originally consisted of 6 trees, however on May 26th 2010 one had to be removed. Two new sites were added in 2010. The first new site consisted of 10 trees along East Shaw lane between Bogue Street and Wilson Road North of the IM Sports East building on the campus of Michigan State University. The Shaw lane site is referred to as (SW) (Figure 3). The SW site is a highly visible corridor along one of the main entrances to the Michigan State University, and so had a level of acceptable appearance that needed to be kept up. As a result one of the SW trees in our study feel below acceptable appearance levels and had to be removed in the spring of 2010, leaving nine trees for the study.

The second site established in 2010 was along Grand River Avenue, between M.A.C, and Bailey Roads, referred to as (GR). GR consisted of seven oaks, five in the Grand River median, and two along the MSU side of Grand River in lawn areas along the side walk (Figure 4). These trees were older than the other sites, being approximately 30 years old. Finally in the fall of 2012, six more trees on the South side of the Michigan State University Main Library were added to the study referred to as (LB) (Figure 5). The following sites, BR1, BR2, FR, SW, AD, and GR all had oaks that were either control trees, or showed varying degrees of scorch severity among the sites. LB is the only site that had trees of the same age and same soil type, yet had only one tree with signs of minor scorch. Sites BR1, BR2, SW, FR, AD, GR are all growing in what is known as a tree lawn, meaning that they are mulched to roughly half the drip line surrounded by turf. Only the trees at LB are void of turf, planted in parking medians behind the MSU library, with the entirety of their soil surface covered with mulch. Due to the high visibility of sites SW and BR, more pruning and spraying of herbicide took place at those sites. During our five years of study, all sites no longer had a yearly layer of mulch added, and there was little spraying of herbicides at any of the campus sites. All sites had the same range of physical and observational measurements.

CHAPTER 2

LITERATURE REVIEW

Leaf scorch, can have many causal agents, some of which are abiotic in nature. Abiotic leaf scorch is a form of leaf scorch common among landscape trees, caused abiotic factors, usually anthropogenic in nature. A known biological agent that causes a different biotic form of leaf scorch is the xylem-limited bacterium, *Xylella fastidiosa*, which causes the disease known as Bacterial leaf scorch of hardwoods (Sinclair & Lyon, 2005; Wang & Omasa, 2012; Guan et al., 2013).

To better understand the causes of leaf scorch is to better understand the many challenges facing successful management of trees in the landscape including; problems of nursery practices, planting depth, tools and equipment used for planting, root girdling, root restriction, diseases, nutritional toxicities and deficiencies, salt buildup, soil resistance and bulk density, and water movement and availability. Keeping this in mind, the study of the causes of abiotic leaf scorch must be both inclusive and complex. Although abiotic leaf scorch is common in the urban landscape, the causes are however poorly understood. Over the past decade there has been an increase in the scientific data that can definitively show cause and effect between nursery and landscape industry practices and tree mortality. “Scorch is common to a number of landscape tree species and appears more frequently on shade-adapted understory species during periods of high sustained temperature” (Swartz et al., 2008). Understanding the causes of severe abiotic leaf scorch and the subsequent tree mortality requires a broadening of our perception of tree growth, development, time and most importantly nursery and landscape industry practices and standards. The effects of improper nursery or landscaping practices are not apparent until a tree matures or the tree experiences some environmental stress. For example, nursery production and landscape planting practices can cause girdling roots (Harris & Day, 2010; Day and Harris, 2008; Wells et al., 2006; Arnold et al., 2005; Day et al., 2001; Shaw, 1977). These girdling roots do not become a problem till the trees roots and trunk grow to sufficient girth to begin constricting one another, and become strangler roots (Watson et al., 1990;

Shaw, 1977). Additionally, poor site preparation, improper planting depth or leaving the burlap sack and cordage in-tacked around a transplanted tree or any number of other factors can cause slow decline and gradually lead to mortality (Arnold et al., 2005; Wells et al., 2006; Day et al., 2001). The Nursery industry, professional landscapers and those who are involved in the growing and planting of trees have become more aware of the potential adverse effect that early nursery and transplanting practices have on long term tree health (Bryan et al., 2006; Watson, 2006).

Leaf scorch is a frequent harbinger of tree decline in urban landscapes and so is a highly visible outward sign of tree health (MacDonald et al., 1993; Day & Harris 2008, Swartz et al. 2008, Gilman et al. 2010, Wang and Omasa, 2012). The symptoms of leaf scorch have previously been recognized as those of a type of water stress (Kozlowski, 1976; Harris et al., 2004). Texts used to diagnosis plant disorders describe leaf scorch as appearing in periods of high temperatures and or dry winds and often marked by a period of high light intensity or temperature, soil freezing, or sustained winds, essentially any environmental condition that either reduced water availability or increases transpiration or both (Harris et al. 2004, Wang and Omasa, 2012). Interveinal margins of leaves exhibit a drying gradient as leaves interveinal areas become dry, papery and dark brown. Browning of the margins may cross over leaf veins and gradually spread back toward the middle of the leaf. This has been interpreted as an effort by the tree to conserve water that is being lost through transpiration by lessening functioning transpirational mechanisms. Newly transplanted trees with improperly established root systems often show signs of scorch within a year and gradually recover, assumedly as the root system recovers from the stress of root damage, differential soil interfaces, or other problems associated with transplanting (Chalker-Scott & Stout, 2008). The commonly held belief is that water loss from transpiration in the leaves was simply occurring faster than the root system could replace (Westcott & Horst, 2001). As symptoms progress, from low, to moderate to heavy scorch, the canopy of the tree will appear increasingly dry and papery. Severely symptomatic leaves may drop, or persist along with a new flush of leaves in mid or late summer (Harris et al., 2004; Wang and Omasa, 2012). Leaf scorch symptoms, caused by abiotic processes, can cause a slow and progressive dieback of terminal twigs, eventually leading to the mortality of entire branches.

In this current study trees were observed to overcome leaf scorch. That is, the underlying reasons for scorch either no longer exist, or the tree through internal processes grows and overcomes the condition. Mortality, dieback, and decline are most evident on trees with reoccurring severe scorch symptoms that become progressively worse year to year.

In order to properly transport water to the leaves, trees need to have a functional root system (Kozlowski, 1976). The challenge in diagnosing causes of root architecture problems are that the system in question may be dysfunctional, but may not be visible, rather hidden underground (Hudler & Beale, 1981). If the tree was not planted at the correct depth in the nursery, and subsequent transplantings resulted in deeper transplanting so that structural support by staking is not needed, then the trees root system undergoes severe stress that leads to long term decline (Single, 2009; Day et al., 2001).

Root systems require oxygen in order to respire. In heavy soils or soils with compaction issues the deeper the roots grow in the soil the less oxygen is available (Ohga & Ikushima, 1970; Weltecke & Gaertig, 2012). Planting a tree too deep, meaning that the soil surface is above the primary root flare, induces stress on the tree, often reducing survival, and growth rate (Bryan et al., 2006). Additionally, in some species these conditions favor formation of a second root collar of adventitious roots above the original root flare (Meilleur, 2008). This second tier of roots grows at the soil surface in response to the lack of oxygen in the deeper soils and is a common feature of plants growing in frequently flooded environments (Kozlowski and Pallardy, 2002). The deeper roots start to run at respitory deficits which lead to stress (Tusler et al., 1998). Further problems occur when the second tier of roots initialize and attempt to spread out radially. Adventitious roots often form on one or more areas of the new soil interface and attempt to spread out radially, usually growing against the trunk. As these roots grow and increase in size they eventually grow and come in contact with the trunk. As the roots and trunk grow they compress the trunk and girdle the tree (Gilman & Grabosky, 2004; Wells et al., 2006). Other landscaping practices, such as the addition of thick layers of mulch on a yearly or bi-yearly basis can have the same effect as a grade change. As the mulch decomposes, it adds to the surface soil layer, burying the root collar and leading to root stress, depending on the sensitivity of the species, hindering establishment and growth (Arnold et al., 2005). Some research suggests that species adapted to wetland habitats are more tolerant to such conditions (Walls et al., 2005). Thick mulch maintains a moist environment around the trunk collar, which may initiate growth of fibrous roots in the mulch layer that may encircle the trunk if mulch thickness is maintained for successive years. Girdling roots may progressively inhibit water transport in the strangled trunk, depending on tree species, aggravating leaf scorch by inducing branch dieback, reducing canopy size and impacting overall health (d'Ambrosio, 1990). This ultimately leads to tree decline, structural failure and death.

Another common site problem leading to a decline in tree health and possibly abiotic leaf scorch is degradation of soils by anthropogenic activities. Urban landscapes often have a complex history were soils are concerned (Urban 2008). The most common soil problem in urban landscapes is compaction (Day et al., 1994; Day et al., 2001; Urban, 2008; Nadezhdina et al., 2012), soil contamination by pollutants such as road salt, other contaminants associated with vehicles (Baligar et al., 1998), and by altered soil structure due to disturbance (Craul, 1992). Soil problems can cause reduced tree vigor by limiting root growth, which can cause trees to have an increase in sensitivity to drought stress. These two factors, reduced root growth, and increased sensitivity to drought stress; are potential components that can affect severity of leaf scorch and decline symptoms.

Most planting sites have been modified to some and in many cases to a severe degree. Soils are modified by grading and construction equipment (Bockheim, 1974). In years past, site disturbance and grade changes were often implemented with little or no attention given to favorable growth conditions for trees (Urban, 2008). Industrial backfill soils, that is soils containing a large component of broken and crushed concrete, brick, asphalt fragments and construction debris compacted by machinery are top dressed with a minimum of favorable topsoil which contains organic matter and nutrients necessary to increase tree vigor. These anthropogenic processes create new soil strata with complex interfaces often blending existing soil textures destroying the natural soil structure (Craul, 1992). Rubble of cobble-sized stones, and gravel or fragments of brick and cement with diameters greater than 2 cm, inhibits root elongation if the open spaces are not filled with sand or soil. Such materials create impenetrable to root growth. The large pores also inhibit water movement from fine pored soils to large pored gap material due to large differences in the matrix potentials of the two substrates. Large differences between the pore sizes and matrix potential of a top soil and those of an adjacent layer of sand can also impede water movement and root growth causing local drought stress on a tree despite proximity to an otherwise adequate supply of soil water (Jim, 1998). Further problems occur with trees that have a large constituent of clay in their root balls, planted in top soils containing loam and organic matter, layered over soils with a largely sand composition. In such a situation the root ball is the last part of such a soil matrix to absorb water and achieve saturation. Even if the root ball is under direct irrigation, the surrounding loam soil will draw water out of the root ball until it achieves saturation, only then allowing the root ball to achieve hydraulic equilibrium with the surrounding soils (Nelms & Spomer, 1983; Chalker-Scott & Stout, 2008).

Soil compaction can cause water stress in trees (Nadezhdina et al., 2012). This water stress is caused by a restriction in root elongation due to root tips not being able to penetrate between soil particles with pores sizes smaller than the diameter of the growing tip of tree roots (< 60 μm) (Coder, 1998). Roots growing under such conditions will alter normal growth by producing more lateral roots (Coder, 1998). Compaction can also cause water stress in the soil where water is present yet unavailable due to soil pores size holding the water too tightly (Jim, 1998). Oxygen diffusion through the soil becomes reduced (< 0.2 $\mu g/cm^2/min$) causing roots to preform cellular respiration at an oxygen deficit as growing roots use oxygen quicker than soil oxygen can be replaced through diffusion (Tusler et al., 1998). Under these various soil condition tree vigor is reduced, leading to long term decline (MacDonald et al., 1993). Roots are estimated to require a concentration of 15% oxygen in soil pores, (the atmospheric concentration of oxygen is 24%), root growth initiation requires 12% oxygen, and active root growth requires a minimum of 5% oxygen (Coder, 1998). Elongation ceases at oxygen levels below 5% (Coder et al., 1998). Soil compaction can reduce soil pore size decreasing soil oxygen to 14% of available atmospheric oxygen levels, causing roots to run at a respitory deficit (Coder, 1998).

Clay soils, or any combination of clay conglomerate soils such as Clay loam and silty clay, are much more easily compacted due to the nature of soil particle. Sand and sandy silt soil is less likely to become compacted, for the same reason (Jim, 1998). The larger particle size in sandy soils can lessen soil compaction, as can seasonal ground freezing that causes shrinking and expansion from “frost heave”. In Northern states such as Michigan, this can occur up to the depth of the “frost line” which can be as deep as 92 to 107 cm (MICHIGAN RESIDENTIAL CODE 2003). This seasonal cycle of soil expansion and contraction helps to break up compaction, as does the activities of soil micro-fauna such as soil insects, detritivores and earthworms. However, soil disturbances and compaction negatively affect soil biota, leaving urban soils essentially lifeless (Bockheim, 1974).

Oaks often exhibit nutrient deficiency symptoms in leaves when soil conditions are unfavorable to root growth or absorption as those described above. In calcareous and high pH soils iron (Fe), zinc (Zn), and manganese (Mn) deficiencies are common which may be expressed in chlorotic leaves with green veins (Marschner, 1995). Species, such as oaks, adapted to more acid soils will not be able to access these nutrients which are also less available at higher soil pH. Manganese becomes available in soils with neutral to acidic pH, optimally pH 6.0 (Lucas & Davis, 1961). Oaks commonly exhibit foliar nutrient imbalances and associated progressive dieback in landscapes. Oaks with nutrient imbalances caused by alkaline soils often show abnormally low Mn and high magnesium (Mg) levels. Rain (pH 4.5-6.5) after running off pavement and sidewalks, urban and suburban parkways, often varies from pH 7.5-8.0 and such runoff has induced Mn deficiency (26-38 μg/g leaf tissue) in white oaks down slope from a parking lot soon after construction (Messenger, 1986). Similarly, cement dust deposits, such as occur in soils adjacent to sidewalks, roads, construction sites, and have been reported to cause such leaf symptoms in oaks and other established trees by increasing soil pH (Rhoads, 1976).

Interactions among the essential microelements also occur. Mn influences the transport of Fe in the leaf, leaves with Mn deficiency may contain higher than normal levels of Fe, furthermore foliar applications of Fe may decrease chlorosis symptoms in the high Fe-low Mn leaves (Messenger, 1983). Fe treatments are often used extensively with oaks but with little perceived benefit (Messenger, 1983). Mn oxidizes Fe converting it into an insoluble form preventing transport in from the leaf (Chapman, 1931). Mn serves as a catalyst for numerous enzymes and is important for chlorophyll function, thereby affecting the efficiency of photosynthesis (Johnson et. al., 1988).

Indirect methods can be utilized to quantify the physiology of scorched plants compared to non-scorched plants. Plant stress can be detected early in plants prior to the expression of damage, such as leaf scorch. For example, hydraulic conductance can be used to assess the efficiency of water transport in trees (Cowan & Milthorpe, 1968; Swartz et al., 2008). This measure can quantify restrictions in xylem flow or indirectly reflect the function of the roots at the soil interface, including parameters of root transport and absorption efficiency (Passioura, 1988). Other means of studying water transport and water stress in trees include leaf xylem pressure potential measured with Scholander pressure chambers (Scholander et al. 1965), or leaf transpiration or stomatal resistance (conductance) measured with a porometer (Boyer, 1985; Waring & Cleary, 1967). Normally, chlorophyll fluorescence changes as a safety method of disposing of excess solar radiation to avoid photo-oxidation damage to the photosynthetic systems, specifically PSII (Naumann et al., 2007). Under stresses such as drought, the safety mechanisms do not function as effectively as normal. Instruments are now available that can detect fluorescence differences indicative of stress. Field differences can then be related to prior laboratory studies of drought stress under controlled conditions. Therefore, measures of chlorophyll fluorescence of attached oak leaves can detect early physiological stress that later may be expressed as scorch damage on leaves. Efficiency of photosynthesis, stomatal conductance, and the other physiological parameters could reveal important information for understanding leaf scorch (Swartz et al., 2008).

Evaluating the impacts of planting depth and progressive root architecture is an important and newly recognized challenge in understanding limitations to root function and tree health (Watson, 2006; Watson et al., 1990; Shaw, 1977; MacDonald et al., 1993; Day & Harris, 2008; Gilman et al., 2010). The capability to non-destructively excavate root systems using supersonic pneumatic tools, such as the 'air-spade' (Anonymous, 2008), has greatly enhanced the ease of diagnosis of root and planting problems in established landscape trees. Furthermore, recommendations for professional management of tree root growth are urgently needed in arboriculture. In order to develop a better understanding of the cause or causes of abiotic leaf scorch a thorough analysis of specific site characteristics and physiological responses compared to the severity of abiotic leaf scorch symptoms is necessary. With so many known causes both individual variables and combinations compounding abiotic leaf scorch, there is often no simple answer, no one factor that can be pointed to, and thus remediated. In this way, finding the cause becomes an academic endeavor, with little real world application for the nursery industry at large which is concerned with how such problems can be fixed. If the cause is not one thing, but a combination of things, then the cure becomes too costly. In the interest of the benefit versus an investment of time and money, often the answer is to remove and replace the scorched tree (Tyler Stevens, city forester: Grand Rapids MI. Pers Com).

If the customers, either a municipality, or private home owner, had a better understanding of expectations for survival and longevity rate and was aware of best practices, then the industry would have to change its practices to meet with the demand for a quality product. In the 2009 Landscape Below Ground III proceedings Jamie Single wrote: "Accepting established methods and compromised quality is no longer necessary or acceptable and the industry must support the research and development currently being undertaken to tackle these age old production problems and progress… However, problems remain. Too many buyers are prepared to accept sub-standard stock and consequently, there is 'over-specification' in an attempt to counter the problem of poor quality. 'Over-specification' has led to the search for perfection, were in nature perfection is not appropriate." This "over-specification" is what landscapers and nursery professional point to as a reason for their lack of enthusiasm for the proposed changes to long implemented practices.

CHAPTER 3

PAPER FOR PUBLICATION

Site Specific Causes of Abiotic Leaf Scorch in Urban Red Oaks *(Quercus rubra)*

ABSTRACT

Abiotic leaf scorch is a common symptom of declining trees in urban landscapes. In order to better understand abiotic leaf scorch in northern red oaks *Quercus rubra*, we investigated the possible physiological problems that may be at the root of this malady. Established trees with a known history of abiotic leaf scorch were systematically evaluated for depth of original root flare (DORF), percent girdling root (%GR), soil compaction (Pen (J/m)), visual scorch rating (VSR), average growth per year (AGY), and average basal area growth rate per year (BAGR) to identify possible correlations between growth, site problems and abiotic leaf scorch. Using the above parameters, oak trees with VSRs of zero to three on the same site and among sites were examined looking for possible correlation between growth, site characteristics and severity of VSR symptoms. Trees infected by bacterial scorch pathogens have been excluded from our studies in order to concentrate on the abiotic causes of leaf scorch. Planting depth (as indicated by DORF) was positively correlated with scorch rating. Basal area growth rate was negatively correlated with DORF. Basal area growth rate was also significantly positively correlated with Pen (J/m). Girdling roots (%GR) were positively correlated to DORF. However, VSR was not significantly correlated with %GR. In an attempt to develop recommendations for preventing and treating the disorder, we highlight the importance of proper planting depth in maintaining tree health and vigor.

INTRODUCTION

The symptoms of abiotic leaf scorch are understood to be caused by a type of water stress (Kozlowski, 1976; Kozlowski, 1985). Literature used to diagnosis plant diseases has described leaf scorch as resulting from periods of excessive temperatures, dry winds, period of high light intensity, or soil freezing. Therefore, any persistent environmental condition that increases transpiration and or hinders water availability may lead to leaf scorch (Kozlowski & Pallardy, 2002; Wang & Omasa, 2012). A better understanding of the underlying causes of abiotic leaf scorch, its association to the decline and death of trees in the urban environment, requires a multifaceted approach taking into considerations the possible effects of planting practices on tree growth and development.

In order to properly transport water to the leaves, trees need to have a functional root system (Kozlowski, 1976). Root systems require oxygen in order to respire. In heavy or compacted soils, the deeper the roots grow the less oxygen is available (Ohga & Ikushima, 1970; Weltecke & Gaertig, 2012). Planting a tree such that the soil surface is above the primary root flare, induces stress on the tree, often reducing survival, and growth rate (Bryan et al., 2006). Additionally, in some species these conditions favor formation of a second root collar of adventitious roots above the original root flare (Meilleur, 2008). This second tier of roots grows at the soil surface in response to the lack of oxygen in the deeper soils and is a common feature of plants growing in frequently flooded environments (Kozlowski & Pallardy, 2002). Further problems occur when this second tier of roots attempt to spread out radially. These adventitious roots often form on one or more areas between the new soil surface and the original root collar and attempt to spread out radially growing against the trunk. As these roots and the trunk expand they grow to touch each other, later compressing the trunk and girdling the tree (Gilman & Grabosky, 2004; Wells et al., 2006). Compounding this stress, root respiration decreases with soil depth due to limited oxygen in the deeper soil (Tusler et al., 1998). The challenges in diagnosing root architecture problems are that the system is hidden underground (Hudler & Beale, 1981). If the tree was not originally planted at the correct depth in the nursery container, and subsequent transplantings resulted in deeper transplanting, then the root system undergoes severe stress that leads to long term decline (Single, 2009; Day et al., 2001). Newly transplanted trees with compromised root systems often show signs of scorch within the year and gradually recover, presumably as the root system recovers from the stress of root damage, differential soil interfaces, or other problems associated with transplanting (Chalker-Scott 2007; Chalker-Scott & Stout, 2008). The commonly held belief is that water loss from transpiration in the leaves is occurring faster than the stressed and damaged root system could replace (Westcott & Horst, 2001).

Nursery production and landscape planting practices can induce girdling roots (Harris & Day, 2010; Day & Harris, 2008; Arnold et al., 2005; Wells et al., 2006; Day et al., 2001; Shaw 1977). Trees with girdling roots do not exhibit a decline in growth until the trees roots and trunk begin constricting each other as both increase in girth, such roots become “strangler roots” (d’Ambrosio 1990; Shaw, 1977). Improper preparation of the site, improper planting depth (MacDonald et al., 1993; Day & Harris 2008; Gilman et al., 2010) and soil compaction can cause tree decline and eventual mortality (Arnold et al., 2005; Wells et al., 2006; Day et al., 2001) when aggravated by girdling roots.

Soil compaction can cause water stress in trees (Nadezhdina et al., 2012) and therefore could have a role in abiotic leaf scorch. This water stress is caused by a restriction in root elongation by root tips being unable to grow between soil particles with pores sizes smaller than the diameter of the growing tip of the root (Coder, 1998). Roots growing under such conditions will alter normal growth by producing more lateral roots (Coder, 1998). Compacted soil can also cause water stress where ample water is present. Compacted soils can make available water unavailable by holding water too tightly by capillary action because of reduced soil pore size (Jim, 1998). Compaction can also hinder normal oxygen diffusion through the soil, resulting in roots having to preform cellular respiration under anoxic conditions, as oxygen is used quicker than it can be replaced through diffusion (Tusler et al., 1998). Under such soil condition tree vigor is reduced, leading to long term decline (MacDonald et al., 1993).

In order to identify the contributing factors in abiotic leaf scorch in Northern red oaks (*Quercus rubra* L.), and to understand the accompanying physiological problems that may cause this common symptom of declining trees in urban landscapes, we examined groups of *Q. rubra* trees with and without leaf scorch and sharing similar age and soil conditions, on the Michigan State University campus. The variables we measured to identify possible correlations between growth, site problems and abiotic leaf scorch included: depth of original root flare (DORF), percent girdling root (%GR), soil compaction (Pen (J/m)), visual scorch rating (VSR), average growth per year (AGY), and average basal area growth rate per year (BAGR). Oak trees with VSRs of zero (no scorch) to three (severe scorch) on the same site and among sites were examined to determine correlations between site variables and presence and severity of VSR symptoms.

METHODS & MATERIALS

Study Sites:

In 2008 three study sites were selected on the campus of Michigan State University. The sites were selected based on observations from previous years of established *Q. rubra*, with a variety of individuals exhibiting different levels of scorch, adjacent to non-scorched oaks of the same age on similar soil types. Additional sites with similar characteristics were added in 2009, 2010, 2011, and 2012 (Table 1). In total, the study consisted of seven sites; four of the sites had trees that were 23 to 28 years old and the trees at the other two sites were between 43 and 48 years old. Tree ages were determined by planting dates as recorded in the Michigan State University campus tree inventory database. Some sites received regular irrigation by subsurface sprinkler systems, while others received only natural rain as irrigation (Table 1). Of the original four sites established in 2008, the first two sites were at the Breslin Center (Jack Breslin Student Events Center) and are referred to as Breslin 1, and 2, or as (BR1) and (BR2). Of the other original four sites, the Oak hill Cemetery in Adrian Michigan was referred to as (AD), and the site on the west side of Farm Lane between East and West bound Shaw Lane (Table 1) was referred to as (FR). Only BR1, BR2, and FR continued to be used yearly in this study. Site AD was dropped from the study after 2009 due to unavailability of travel funds.

Two additional sites were added in 2010: Shaw Lane (SW) consisted of 9 trees along East Shaw Lane (Table 1) and Grand River Avenue (GR) near downtown East Lansing which consisted of seven oaks, five in the Grand River median and two along the south side boulevard of turf. SW and GR trees were older than those on other sites, being approximately 48 years old. For logistical reasons only 1 of the 7 trees at site GR could be evaluated for any below ground parameters. Six more trees on the South side of the Main Library (LB) planted in parking medians were included in the study in fall 2012 (Table 1). The following sites, BR1, BR2, FR, SW, AD, and GR each contained trees with varying degrees of leaf scorch from 0-3. LB was the only site that had only one tree showing signs of mild scorch, of six. Sites BR1, BR2, SW, FR, AD and GR had been mulched to roughly half their drip line surrounded by turf. The trees at LB were mulched to their drip lines and devoid of surrounding turf. All trees on all sites were subjected to the same range of physical and observational measurements.

Above Ground Parameters

Visual Scorch Rating (VSR):

Severity of scorch symptoms on leaves was visually evaluated based on percentage area of necrotic tissue per leaf and a rating of no scorch, low, moderate and high scorch: VSR of 0,1,2,3 respectively (Figure 6). A VSR was assigned to each study tree. The visual rating was standardized by having the same person categorize all leaves to ameliorate potential human bias. Since the scale was based on visual observation and each category has a gradient, it was important to have examples of each category of leaf scorch visually available for reference, and to perform and record a complete set of multiple observations at a time (Figure 6). Leaves were photographed each month from May through October. Each photograph was assigned a VSR and a yearly average for each tree was calculated.

Average Growth per Year (AGY):

Tree height (m) was measured using a Trupulse® (Laser Technology, Inc. Centennial, Colorado USA) 200 Rangefinder/hypsometer and age was established by using archived information on planting history. Then, average growth per year was calculated by dividing height by age of each tree.

Average Basal Area Growth Rate (BAGR):

Diameter at breast height was measured at 1.4m from the base of the tree using a Lufkin® diameter tape, and cross sectional area in cm^2 was calculated as: $A = \pi r^2$. Then, average cross sectional area growth rate was calculated as A/tree age.

Soil Compaction (Pen (J/m)):

Soil compaction was determined using a dynamic mechanical cone penetrometer (Herrick & Jones, 2002). The degree of compaction of soil in the root zone of the experimental trees was quantified by determining soil resistance (impedance) to penetration using a penetrometer. Soil resistance measurements were determined with a custom built dynamic mechanical cone penetrometer designed and manufactured according to (Herrick & Jones, 2002). Measurements were made in three separate cardinal directions and midway to the drip line for each tree. The depth of penetration, Pd (m), following 10 drops of the 2 kg weight from a height of 40 cm (velocity of 2.8 m/sec and kinetic energy of 7.84 Joules per drop) for a total kinetic energy of 78.4 J was measured. The soil resistance, Rs (in Newtons) = Ws/Pđ where Ws is the work done by the soil in impeding the penetration (in Joules), and Pđ is the mean distance (m) the penetrometer traveled through the soil during the three measurements (Herrick & Jones, 2002); herein, Rs = 78.4 J/Pđ." The same data was collected for sites BR1, BR2, FR, and AD, as previously reported in (Swartz et al., 2008). The same method was used to collect data for sites SW, GR, and LB.

Soil pH:

Soil assays to determine total pH were performed by the Soil and Plant testing Laboratory Plant at Michigan State University using standard methods.

Identification of Biotic Stress:

In order to avoid confusing leaf scorch induced by (biotic) pathogens with abiotic leaf scorch, the presence and quantification or absence of the pathogen *Xylella fastidiosa* was determined using species-specific oligonucleotide primers and machinery and reagents of the quantitative polymerase chain reaction DNA amplification methodology (Real-time PCR or qPCR) for the SYBR® Green protocol (Applied BioSystems Inc., Foster City, CA). DNA was extracted from petiole xylem tissues of symptomatic and symptomless leaves and three separate extractions and amplifications were evaluated for each tree sample and compared to a positive pathogen DNA control with calibrated dose detection curve, a negative DNA-free control, and a plant gene quantitative control (Adams et al. 2011).

<u>Below Ground Parameters</u>

Air Spade Excavation:

The preservation of all trees used in this study was essential so a nondestructive method of soil removal was needed. This was accomplished by using an "Air-SPADE®" series 2000 (Guardair Corp., Chicopee MA USA). This tool releases high pressure air at a rate of 6.2 bars and flow rate of $4.2m^3$/min at the nozzle to remove soil and debris from a root system in a way that does not damage even fine roots (Anonymous, 2008). Evaluations were made after removing soil from around the tree trunks to a minimum distance of .5 m and depth of 30 cm. Sites BR1, BR2, FR, SW, LB, and AD had extensive root evaluations to determine planting depth, presence or absence of planting hardware, (wire cages, steel banding, and cordage) and percent girdling roots. Girdling root severity was determined using a method to evaluate approximate root flare present at planting. This was measured in relation to the surface of the current soil line. A 4 foot (1.3 m) long metal straight edge ruler was placed on the soil surface to span the excavated root flare area; a second ruler was used to measure depth vertically from the bottom of the straight edge to the top of the original root flare. Due to site constraints, the evaluations were only preformed on one of the seven oak trees at the GR site.

Planting Depth:

Following air spading, the depth of the original root flare was measured in relation to the surface of the current soil line. A 4 foot (1.3 m) long metal straight edge ruler was placed on the soil surface to span the excavated root flare area; a second ruler was used to measure depth vertically from the bottom of the straight edge to the top of the original root flare. In some instances excessive mulch and soil was mounded around the base of the tree greater than the surrounding soil height, resulting in a planting depth that could not be determined using the straight edge. For several weeks after excavation and exposure this depth was visibly imprinted onto the bark as a persistent mineral ring on the tree trunk. This allowed us to visually confirm the planting depth of each tree, allowing an exact measurement of depth (Figure 7).

Percent Girdling Root (%GR):

Determination of the extent of root girdling was achieved by measuring the length of the arc of the trunk that had a girdling root growing into it, divided by the total circumference of the trunk. This gave the percent of the trunk that was being girdled by that individual root. By adding up these numbers a total percent girdling root could be measured as a percentage of trunk circumference being compressed by encircling “Strangler roots”.

Statistical Analysis:

All statistical analyses were conducted using Sigmaplot version: 12 (Systat Software, Inc. San Jose, CA USA). Pearson product moment correlation matrix was performed comparing depth of original root flare (DORF), percent girdling root (%GR), soil resistance (Pen(J/m)), visual scorch rating (VSR), average growth per year (AGY), and average cross sectional growth rate per year (BAGR),

RESULTS

Among all of the tree and site variables examined VSR alone had a positive (*r*) = 0.33 correlation with DORF (*p*) = 0.015, indicating that increasing planting depth resulted in grater VSR. BAGR compared to DORF had a negative correlation coefficient of (*r*) = -0.348 and a statistically significant (*p*) = 0.0192. The average basal area growth rate per year (BAGR) compared to soil penetrometer/soil resistance (Rs) in Newtons (N) = Ws/Pđ, (Pen (J/m)) had a positive correlation coefficient of (*r*) = 0.469 and a highly statistically significant (*p*) = 0.000883. DORF and GR showed a correlation coefficient (*r*) = 0.329, and was statistically significant with (*p*) = 0.0173 (Table 2). No significant difference was observed between soil pH and VSR,

DISCUSSION

Abiotic leaf scorch is a common condition in urban trees resulting in a loss of vigor (Levitt 1980) which has received little attention as to the cause and possible mitigation. The goal of this research was to develop a better understanding of the cause or causes of abiotic leaf scorch by analyzing specific site characteristics and physiological responses compared to VSR. Chlorosis in urban red oaks can be induced by high soil pH (Kozlowski & Pallardy, 2002), which can limit availability of certain nutrients. However, in this study there was no correlation between VSR and soil pH. We identified several potential causes for abiotic leaf scorch and investigated if there is a correlation between these physical factors and the degree to which trees will scorch. One factor that can impact tree vigor is historic planting depth and progressive root architecture that develops in response to planting depth (Watson 2006; d'Ambrosio 1990; Shaw 1977; MacDonald et al., 1993; Day & Harris 2008; Gilman et al., 2010). Trees in this study with high VSR show a significant positive correlation with DORF (Figure 8), meaning that the deeper a tree is planted above the original root collar the more abiotic leaf scorch symptoms it will have. Since abiotic scorch is linked to tree decline and mortality, greater planting depth means decreased vigor, resulting in decline and mortality.

Previously published studies report the detrimental effects of planting trees too deep (MacDonald et al., 1993; Wells et al., 2006; Arnold et al., 2005; Day & Harris, 2008; Gilman et al., 2010; Harris, & Day, 2010). Our research supports these previous reports and adds the statistically significant correlation between DORF and the occurrence of abiotic leaf scorch (Figure 8). Additionally, our results support previous reports that planting depth significantly correlates with the formation of girding roots (Figure 9). However, it is interesting that we did not find a significant correlation in the study between %GR and VSR (Table 2).

The data presented here support the hypothesis that trees planted too deep also grow a new root collar closer to the surface as represented by a greater %GR. This growth results in roots developing from a few spots on the buried trunk that attempt to spread out tangentially resulting in the formation of girdling roots (Gilman & Paz 2009). The roots below this second collar do not have the oxygen they need to respire, and run at an oxygen deficit leading to stress, reduced vigor, and reduced root growth in some tree species (Gilman & Paz 2009). The reduction in root growth is a response to increased DORF. This reduces growth rate as buried roots need to be maintained while running at an oxygen deficit (Kozlowski, 1985), combine with the need for the tree to invest more energy to generate a new root collar could be the reason for the significant reduction of BAGR (Figure 10).

Another abiotic factor impacting tree growth in urban environments is soil compaction. (Nadezhdina et al., 2012) reported soil compaction as a cause of water stress in trees. Compaction causes a restriction in root elongation since growing root tips cannot penetrate the compacted soil characterized by pores sizes smaller than the diameter of the growing tip of tree roots (Coder, 1998). This results in altered root growth as the tree produces more lateral roots (Coder, 1998) which can also lead to the formation of girdling roots. Compacted soil can hinder normal oxygen diffusion through the soil, resulting in roots having to perform cellular respiration under anoxic conditions, as oxygen is used quicker than it can be replaced through diffusion (Tusler et al., 1998). Under such soil condition tree vigor is reduced, leading to decline (MacDonald et al., 1993). Our results support this body of knowledge as we report a highly significant correlation p=0.0009 between Pen (J/m) and BAGR (Figure 11). Hence, a higher compacted soil requires more force (J) to penetrate one meter (m), or highly compacted soils offer greater resistance to penetration.

CONCLUSION

The purpose of this research was to look for possible correlations between severity of VSR, tree growth and site characteristics. We have shown that trees in our study sites with smaller BAGR are planted in more compacted soils and are planted deeper compared to trees with larger BAGR among sites and on the same sites. The data also shows that the trees which were planted the deepest have the most severe VSR ratings and also have the highest %GR. We conclude based on the results of this study that excessive planting depth induces abiotic leaf scorch and although planting depth also correlated with the formation of girdling roots; no correlation was shown between girdling roots and abiotic leaf scorch. Planting depth is crucial to establishment of healthy trees. If trees were planted too deep at the time final installation in the landscape, then we recommend that the excess soil be removed to expose the root flare, and any girdling roots not already fused to the trunk be removed.

APPENDICES

APPENDIX A

PHYSIOLOGICAL DATA

INTRODUCTION

Leaf scorch can have many causal agents, many of which are abiotic in nature. These causes are however poorly understood. Since leaf scorch is often a symptom of decline and mortality, my research has focused of leaf scorch as a means of quantifying tree health by looking at differing levels of leaf scorch and comparing scorch to planting practice, tree physiology, soil oxygen and soil nutrient data that were collected over a six year period. This chapter will address the following question: What are the possible correlations between abiotic leaf scorch and physiological growth responses and is there a correlation between soil compaction, soil oxygen content and abiotic leaf scorch?

Understanding the causes of tree mortality requires a broadening of our perception of tree growth. Leaf scorch is a frequent harbinger of tree decline in urban landscapes and so is a highly visible outward sign of tree health; this problem was first described in (Swartz et al., 2008; Wang & Omasa, 2012) and in Chapter 2: Literature Review.

To better understand the causes of leaf scorch is to better understand the many challenges facing successful management of trees in the urban landscape including; problems of nursery practices, planting depth, tools and equipment used for planting, root girdling, root restriction, diseases, nutritional toxicities and deficiencies, salt buildup, soil resistance and bulk density, and water movement and availability. Leaf scorch symptoms, caused by abiotic processes, can be expressed in trees as a slow and progressive dieback of terminal twigs, eventually leading to the mortality of entire branches. Some trees have been observed to overcome leaf scorch (personal observation). That is, the underlying reasons for scorch were either seasonal or the tree through internal processes grows out of the condition. Mortality, dieback, and decline are most evident in trees with reoccurring severe scorch symptoms that become progressively worse year to year (Swartz et al., 2008).

In order to properly transport water to the leaves, trees need to have a functional root system (Kozlowski, 1976). Root systems require oxygen in order to respire. Oxygen availability decreases with increasing soil depth (Ohga & Ikushima, 1970). In heavy soils or severely compacted soils less oxygen is available to support root respiration compared to non-compacted soils (Ohga & Ikushima, 1970; Weltecke & Gaertig, 2012). Planting a tree too deep, meaning that the soil surface is above the primary root flare, placing the existing roots in a zone of reduced oxygen content which induces stress on the tree, often reducing survival, and growth rate (Bryan et al., 2006).

A common site problem is degradation of urban soils by anthropogenic activities, often leading to complex soil strata (Urban, 2008). These new soil strata, often blend existing soil textures which destroy the natural soil structure (Craul, 1992). Rubble of cobble-sized stones, and gravel or fragments of brick and cement with diameters greater than 2 cm can hinder root elongation. The large pores also inhibit water movement from fine pored soils to large pored material due to large differences in the matrix potentials of the two substrates, causing local drought stress (Jim, 1998).

Soil compaction which is the most common soil problem in urban landscapes (Day et al., 1994; Day et al., 2001; Urban, 2008; Nadezhdina et al., 2012), can limit water and oxygen availability to tree roots (Nadezhdina et al., 2012; Tusler et al., 1998). Compaction can also cause water stress in the soil where water is present yet unavailable due to soil pores size holding the water too tightly (Jim, 1998). Oxygen diffusion through compacted soils is reduced ($< 0.2\ \mu g/cm^2/min$) causing roots to preform (anerobic?) cellular respiration at an oxygen deficit as the respiration rate of roots use oxygen quicker than soil oxygen can be replaced through diffusion (Tusler et al., 1998). Under these various soil conditions tree vigor is reduced, leading to long term decline (MacDonald et al., 1993). Some estimates of

root requirements for oxygen and concentration of oxygen in soil pores are in the range of 15% oxygen, (24% oxygen is the above ground, atmospheric concentration), root growth initiation requires 12% oxygen, and active root growth requires a minimum of 5% oxygen (Coder, 1998). Elongation ceases at oxygen levels below 5% (Coder, 1998). Soil compaction can reduce soil pore size 14% of available atmospheric oxygen levels, which causes roots to run at a respiratory deficit (Coder, 1998).
Clay soils, or any combination of clay conglomerate soils such as clay loam or silty clay, are much more easily compacted due to the nature of soil particle. Sand and sandy silt soils are less likely to become compacted, for the same reason (Jim, 1998). The larger particle size in sandy soils can lessen soil compaction, as can seasonal ground freezing that causes shrinking and expansion from "frost heave". This seasonal cycle of soil expansion and contraction helps to break up compaction, as does the activities of soil micro fauna such as soil insects, detritivores and earthworms.

Oaks often exhibit symptoms of nutrient deficiency in leaves when soil conditions are unfavorable to root growth or absorption of water, nutrients or oxygen as described above. In calcareous and high pH soils iron, zinc, and manganese deficiencies are common which may be expressed as chlorotic leaves with green veins (Marschner, 1995). Interactions among the essential microelements also occur. Mn influences the transport of iron (Fe) in the leaf, leaves with Mn deficiency may contain higher than normal levels of Fe, furthermore foliar applications of Fe may decrease chlorosis symptoms in the high Fe-low Mn leaves (Messenger, 1983). Fe treatments are often used extensively with oaks but with little perceived benefit (Messenger, 1983). Mn oxidizes Fe converting it into an insoluble form preventing transport from the soil into the leaf (Chapman, 1931). As a catalyst for numerous enzymes and an important structural element of chlorophyll Mn affects the efficiency of photosynthesis (Johnson et. al., 1988).

Indirect methods can be utilized to quantify the physiological responses of scorched plants compared to non-scorched plants. For example, hydraulic conductance can be used to assess the efficiency of water transport in trees (Cowan & Milthorpe, 1968; Swartz et al., 2008). This measure can quantify restrictions in xylem flow or indirectly reflect the function of the roots at the soil interface, including parameters of root transport and absorption efficiency (Passioura, 1988). Other means of studying water transport and water stress in trees include leaf xylem pressure potential measured with a Scholander type pressure chambers, leaf transpiration or stomatal resistance (conductance) measured with a porometer (Boyer, 1985; Waring & Cleary, 1967). Plant stress can be detected early in plants prior to the resulting expression of damage, such as leaf scorch. Normally, chlorophyll fluorescence changes as a safety method of disposing of excess solar radiation to avoid photo-oxidation damage to the photosynthesis system, specifically PSII (Naumann et al., 2007). Under stresses such as drought the safety mechanisms do not function as effectively as normal; and, instruments are now available that can detect fluorescence differences indicative of stress. Field differences can then be related to prior laboratory studies of drought stress under controlled conditions. Therefore, measures of chlorophyll fluorescence of attached oak leaves can detect early physiological stress that later may be expressed as scorch damage on leaves. Efficiency of photosynthesis, stomatal conductance, and the other physiological parameters could reveal important information for understanding leaf scorch.

METHODS & MATERIALS

Study Sites:

As described in chapter 3:

<u>Above Ground Parameters</u>

Visual Scorch Rating:

As described in chapter 3:

Identification of Abiotic Stress:

As described in chapter 3:

Leaf Temperature/Scholander Experiment:

In 2012, a series of three tests were done at sites BR1 and BR2, comparing scorch and none scorch tree, and the possible relationship between leaf temperature and leaf pressure potential (LpP). In June, July, and September of 2012, leaf temperature data were collected using an Extech® Instruments, (Extech® Instruments Nashua, NH U.S.A) InfaRed thermometer model 4252, IR gun (+or- 2% of reading), from ten different leaves in each of the six test trees. Of the six trees, three had a history of scorch, and three were non-scorched trees. General weather information was collected such as ambient temperature, collected by hanging a mercury thermometer in the under canopy of a nearby control tree, allowing it to acclimate for a minimum of 15 minutes, and taking a measurement before and after site work was initiated, along with cloud cover conditions. In addition, leaves were also collected from each of the test trees; leaves from each tree were kept in individual labeled bags in a cooler with frozen ice packs to preserve freshness. For this experiment the leaves were not transported to the lab to obtain stomatal conductance data, rather the Scholander pressure chamber, Model 600, (PMS Instrument Company Albany, OR. USA) and the nitrogen tank were transported to the BR test site so that Scholander readings could be taken in the field.

<u>Below Ground Parameters</u>

Soil Compaction/Chemical Analysis:

For sites BR1, BR2, FR, and AD, as previously reported in (Swartz et al., 2008). The same method was used to collect data for sites SW, GR, and LB.

“Soil assays to determine the proportion of sand, silt and clay, cation exchange capacity (CEC) and total pH were performed by the Soil and Plant testing Laboratory Plant at Michigan State University using standard methods. The degree of compaction of soil in the root zone of the experimental trees was quantified by determining soil resistance (impedance) to penetration using a penetrometer. Soil resistance measurements were determined with a custom built dynamic mechanical cone penetrometer designed and manufactured according to (Herrick & Jones, 2002), base area of the cone was 323 mm^2. A dynamic cone penetrometer was chosen because they tend to yield consistent results and the measurements are more repeatable because the force applied does not vary (Jones & Kunze, 2004). Measurements were made in three separate cardinal directions and midway to the drip line for each tree. The depth of penetration, P_d (m), following 10 drops of the 2 kg weight from a height of 40 cm (velocity of 2.8 m/sec and kinetic energy of 7.84 Joules per drop) for a total kinetic energy of 78.4 J was measured. The soil resistance:

$$R_s \text{ (in Newtons)} = W_s/P_d \qquad \text{(Eq 1)}$$

where W_s is the work done by the soil in impeding the penetration (in Joules), and P_d is the mean distance (m) the penetrometer traveled through the soil during the three measurements (Herrick & Jones, 2002); herein, R_s = 78.4 J/P_d."

Additional soil data was collected for sites GR, SW, and LB in May of 2013. These include soil nutrient/pH and soil Penetrometer measurements. Soil samples were collected using a hand shovel collected from next to the tree trunk, mid-point between the base of the tree and the drip line, and one from just inside the drip line in one of four cardinal direction determined randomly. These data were added to the existing data from the previous year's study for use in Pearson product moment correlation matrixes.

Air Spade Excavation:

Sites BR1, BR2, FR, SW, LB, and AD had extensive root evaluations to determine planting depth, presence or absence of planting hardware, (wire cages, steel banding, and cordage) and percent girdling roots. Girdling root severity was examined using a method that was originally described in

(Swartz et al., 2008). Due to site constraints, the before mentioned evaluations were only preformed on one of the seven oak trees at the GR site. Evaluations were made after removing soil from around the tree trunks to a minimum distance of .5 m and depth of 30 cm. The preservation of these trees was of paramount importance, thus a nondestructive method of soil removal was needed. This was accomplished with a supersonic pneumatic system of compressed air know in the nursery industry as an "Air-SPADE®" series 2000 (Guardair Corp., Chicopee MA USA). This tool releases high pressure air at a rate of 6.2 bars and flow rate of 4.2m^3/min at the nozzle to remove soil and debris from a root system in a way that does not damage even fine roots (Anonymous, 2008). After study, photographing, and measurements were taken, the trees were assessed on an individual basis to determine if the soils should be replaced. In some cases of severe planting depth, steps were taken to alter the planting depth to aid the future growth and development of the trees. In some cases the original soil was reapplied so the root flare could be at an optimal depth as determined based on guidelines for best management practices (Watson, 2006).

Depth of Original Root Flare (DORF):

Following air spading, the depth of the original root flare (DORF) (approximate flare present at planting) was measured in relation to the surface of the current soil line. A 4 foot (1.3 m) long metal straight edge ruler was placed on the soil surface to span the excavated root flare area; a second ruler was used to measure depth vertically from the bottom of the straight edge to the top of the original root flare (Figure 12). This depth was also visibly imprinted onto the unwashed bark of the excavated tree trunk and persisted for several months after excavation.

Percent Girdling Root (%GR):

A measure employed for determination of the extent of root girdling was the ratio of the trunk circumference at soil line divided into the visually estimated percentage of circumference compressed by an encircling root, according to the definitions stated above (Swartz et al., 2008). A relative value was calculated as a measure of the percent of girdle (%GR).

Iron Rods:

Soil oxygen levels and variations in soil strata were measured by driving iron rods into the ground, and later retrieval for visual inspection (Hodge & Knott, 1993). Three iron rods each made of high carbon steel from Alro steel (Alro Corp. Jackson, MI USA), 0.5 cm wide and 60 cm long, were driven into the ground around each of the test trees at each site in one of the four cardinal directions North, South, East, or West as determined by a compass, in random order. One rod was placed between 30 and 40 cm of the tree, the second at the half the distance from the trunk to the drip line of the tree, and the third at the drip line. Iron rods were left in the ground at sites BR1, BR2, and FR. Sites BR1 and BR2 had Iron rods installed between July 22nd and 28th 2008. Iron rods were also installed at site FR on August 8th 2008. The rods were left in the ground over the winter and removed the following spring starting on March 20th 2009. Iron rod removal was concluded at site FR on April 22nd. Each of the iron rods was located using maps and a metal detector. Rods where removed, cleaned with a soft bristle brush wiped with a shop towel, placed on a white back ground with an identification tag, and photographed for later visual inspection. Each of the rods was examined and a rust rating of no rust (0), mild rust (1) or severe rust (2) was assigned to each inch of the rod. Each rod was also divided into three soil profiles of shallowest 20 cm, middle 20 cm, and deepest 20 cm. Average rust ratings where compiled for each depth (Figure 13).

Soil Cores:

In 2009 soil sampling was done at sites BR1, and BR2. Transects were laid out across the two plots, with a core taken every ten feet across tree transects (Figure 14) using an AMS (AMS, Inc. American Falls, ID USA) Basic Soil Sampling Kit, cores were removed using a Sand Auger, 3 ¼ inch (8.3 cm) diameter, thread-on stainless steel Auger bucket, attached to a 4 foot (122 cm) thread-on extension, which was attached to an AMS thread-on cross handle. Coring was done to a depth of 91 cm, or until the corer reached an impediment that could not be cleared. Cores where extracted, removed, placed in line next to the extraction hole, photographed, and three soil samples taken, one form the top, middle, and bottom of the core (Figure 15). Soil analysis to determine pH, and soil nutrient content was performed by the Soil and Plant testing Laboratory at Michigan State University using standard methods. Further soil cores were collected in May 2013 to determine the depth of the topsoil layer at sites BR1 and BR2 for comparison to iron rod data from the shallowest 20cm. Using the original map of iron rod placement, each tree at the two BR sites had three cores taken with a 21 inch AMS® soil probe, in as close an approximation to the placement that the original iron rods were inserted in the ground. Soil samples were extracted, visually assessed for soil texture, and depth of top soil layer was measured in centimeters with a ruler (Figure 16). In this way a field average was collected for later comparison.

Statistical Analysis:

All statistical analyses were conducted using Sigmaplot version: 12 (Systat Software, Inc. San Jose, CA USA).

Correlation Matrix of Physical Properties:

Pearson product moment correlation matrix was performed comparing visual scorch rating, tree height in meters, diameter at breast height cm, soil penetrometer measurements or soil resistance in Newtons = W_s/P_d, depth of original root flare cm, and percent girdling root.

Iron Rods/Soil Oxygen:

Pearson product moment correlation matrix comparing visual scorch rating to soil oxygen levels was performed to analyze these data.

Leaf Temperature/Scholander Experiment:

A Shapiro-Wilk's t-test was performed to show normality with a Mann-Whitney rank sum test was used to compare LpP data for scorched and non-scorched tree. Next a Shapiro-Wilk's t-test for normality was run using leaf temperature averages as the dependent variable. Next a Pearson product moment correlation matrix was used to compare visual scorch rating, leaf temperature, and LpP averages.

Photosynthetic Efficiency, PSII Efficiency:

Pearson product moment correlation matrix was performed comparing photosynthetic efficiency data averages collected in 2008, 2009, 2010 across all sites and visual leaf scorch ratings.

Stomatal Conductance, Leaf Gas Exchange:

Pearson product moment correlation matrix was performed comparing stomatal conductance data averages collected in 2008, 2009, 2010 across all sites and visual leaf scorch ratings.

Scholander Pressure Chamber, Leaf Pressure Potential (LpP):

Pearson product moment correlation matrix was performed comparing Scholander pressure chamber data averages collected in 2008, 2009, 2010 across all sites and visual leaf scorch ratings.

RESULTS

Correlation Matrix of Physical Properties:

A visual scorch rating of 0-3 (VSR) was compared to height in meters, diameter at breast height (DBH) in centimeters, soil penetrometer resistance measurements Pen (J/m) where (R_s) in Newtons = W_s/P_d, depth of original root flare (DORF) in centimeters, and percent girdling root (%GR), collected for each tree at each site. VSR compared to DBH had a negative correlation (r) = -0.282 and was statistically significant with a (p) = 0.0291. VSR and DORF had a positive correlation coefficient of (r) = 0.316, and showed statistical significance with (p) = 0.0211. Height and DBH had the strongest correlation coefficient with an (r) = 0.820, and the highest statistical significance of (p) = 1.042E-015. The second and third highest correlation coefficient was height and Pen (J/m) with an (r) = 0.469, and a (p) = 0.000158, followed by DBH and Pen (J/m) with (r) = 0.454 and (p) = 0.000269. DORF and %GR showed a low correlation coefficient (r) = 0.307, yet was statistically significant with (p) = 0.0256. Non-significant data were VSR compared to height, Pen (J/m), and %GR with (r) = -0.131, -0.00106, 0.233 and (p) = 0.319, 0.994, and 0.0926 respectively. Height compared to DORF and %GR both showed negative correlation coefficients with (r) = -0.0699 and -0.211, with (p) = 0.619 and 0.129 respectively. DBH compared to DORF and %GR had negative correlation coefficients of (r) = -0.251 and -0.124, with (p) = 0.0702 and 0.376 respectively. Lastly, Pen (J/m) compared to DORF and %GR had negative correlation coefficients of (r) = -0.0596 and -0.147, with (p) = 0.672 and 0.294 respectively (Table 3).

Iron Rods/Soil Oxygen:

A correlation matrix comparing visual scorch rating to soil oxygen levels in the shallowest 20 centimeters of the iron rods and top soil depth was non-significant.

Leaf Temperature/Scholander Experiment:

Scholander pressure chamber data failed to show normality. Scholander pressure chamber or leaf pressure potential (LpP) averages were compared to LpP data for scorched and non-scorched tree. The test was non-significant based on median comparison of the scorched and non-scorched trees (p) = 0.353. Next a Shapiro-Wilk's t-test for normality using leaf temperature (LT) averages as the dependent variable was run, which showed there is a significant difference between mean values for LT between scorch and non-scorch trees (p) = 0.002. Next a correlation matrix was used to compare VSR, LT, and LpP averages. The comparison of VSR and LT showed a positive correlation coefficient (r) = 0.634 and a strong statistically significant (p) = 0.00469. There was also a somewhat strong negative correlation coefficient

(r) = -0.532 and strong statistically significant correlation (p) = 0.023 between LT and LpP. There was no correlation between VSR and LpP with (r) =0.188 and (p) = 0.454 (Table 4).

Photosynthetic Efficiency, PSII Efficiency:

Pearson product moment correlation matrix was performed comparing photosynthetic efficiency data averages collected in 2008 (Photo 2008), 2009 (Photo 2009), and 2010 (Photo 2010) respectively, across all sites and VSR. There was a non-significant relationship between VSR and Photo in all years. Also there was a non-significant relationship when 2008 was compared to 2009 or 2010 photosynthetic efficiency data sets. There was a negative correlation coefficient (r) = -0.517 and an extremely strong significant similarity between the 2009 and 2010 photosynthetic efficiency data sets with (p) = 0.000235 (Table 5).

Stomatal Conductance, Leaf Gas Exchange:

Pearson product moment correlation matrix was performed comparing stomatal conductance data averages collected in 2008 (Cond 2008), 2009 (Cond 2009) and 2010 (Cond 2010) respectively, across all sites and VSR. There was a non-significant relationship between VSR and Cond in all years. Also there was a non-significant relationship when Cond 2008 was compared to Cond 2009 or Cond 2010 data sets. There was an extremely strong correlation between the 2009 and 2010 stomatal conductance data sets with a positive correlation coefficient of (r) = 0.567 and an extremely strong significant similarity between the 2009 and 2010 stomatal conductance data sets with (p) = 0.0000496 (Table 6).

Scholander Pressure Chamber, Leaf Pressure Potential (LpP):

Pearson product moment correlation matrix was performed comparing Scholander pressure chamber data averages collected in 2008 (Schol 2008), 2009 (Schol 2009) and 2010 (Schol 2010) respectively, across all sites and VSR. There was a non-significant relationship between VSR and Schol in all years. Also there was a non-significant relationship between the Schol 2008 and Schol 2010 data sets, with similar results when Schol 2009 was compared to the Schol 2010 data. There was an extremely strong correlation between the 2008 and 2009 Scholander pressure chamber data sets with a positive correlation coefficient of (r) = 0.422 and strong significant similarity between the 2008 and 2009 Scholander pressure chamber data sets with (p) = 0.00924 (Table 7).

DISCUSSION

Previously published studies report on the detrimental effects of planting trees too deep (MacDonald et al., 1993; Wells et al., 2006; Watson et al., 2006; Arnold et al., 2005; Day & Harris, 2008; Gilman et al., 2010; Harris & Day, 2010). My previous research as reported in chapter 3, supports statistically significant correlation between planting depth and the occurrence of abiotic leaf scorch, additionally, I reported that planting depth correlates with the formation of girding roots (Table 3). However, it is interesting that I did not find a significant correlation in my study trees between the presence of girdling roots and leaf scorch (Table 3). Thus, I conclude based on the results of this study that excessive planting depth induces abiotic leaf scorch and that girdling roots do not cause abiotic leaf scorch.

In order to understand the possible role that water relations play in inducing abiotic leaf scorch from physiological perspective, I conducted two experiments using scorched and non-scorched trees comparing VSR to temperature, and VSR to Scholander pressure chamber. In the leaf temperature study, as VSR increases so does leaf temperature. This can be explained by an increase in the leaf surface becomes scorched, there a fewer functional stomata reducing the potential for evapotranspiration, resulting in less evaporative cooling of the leaf, thus the observed rise in leaf temperature. This being said, the rise in temperature although statistically significant is only 6°C, ranging from 25° C - 27°C in non-scorched trees to 28°C – 31°C in scorched trees. With the ambient temperature at 30°C the scorched leaves were only 1°C higher. Peak photosynthesis occurs at 35°C and in order to negatively affect photosynthetic efficiency in health leaves 38°C has to be exceeded for more than 7 days (Janka et. al., 2013). The underlying cause may be the result of dysfunctional xylem transport (hydraulic conductivity), physical damage to root morphology, damage to tree anatomy from stem girdling roots, reduced soil water holding capacity, soil aeration, nutritional stress or some combination of all these factors.

Permanent damage to leaf tissue which is known as heat killing temperature for most plants is between 49°C and 70°C and must be sustained for 10 minutes to an hour in some cases (Levitt, 1980). None of the trees, either scorched or non-scorched, were exposed to those temperatures, so it is not likely that the temperature in the scorched leaves, though higher than the ambient temperature was causing any damage to the leaves. In this study, I report a negative correlation between LpP and leaf temperature (Table 8).

Leaf temperature readings were taken at midday, on bright sunny days with little to no cloud cover. Midday is the time of day when plants are most actively transpiring, and even trees that are not experiencing water stress can have a difficult time keeping up with the water demands of foliage, and will at times close some or all their stomata to regulate this water loss (Figure 17). Trees that are exhibiting scorch have lower leaf xylem pressure, conductance and transpiration (Swartz et. al., 2008) and are more likely to have closed or fewer functioning stomata at any point during the day, of which midday would be the most likely time to find the greatest amount of functioning stomata closed. Leaves with closed stomata would have little to no evaporative cooling taking place. Also, leaves with a high VSR have a higher percent of dark colored material that would absorb light as heat. Scorched leaves would then have a lower leaf pressure potential than non-scorched leaves that have open stomata operating under greater transpirational load with higher leaf pressure potential. A negative correlation between Scholander and VSR is shown in (Table 7) which could be interpreted as showing the closing of stomata at midday in order to control transpirational loss in already water stressed leaves. Abiotic leaf scorch symptoms represent a sensitivity of the tree to periodic water stress (Kozlowski, & Pallardy 2002). Thus, leaf scorch limits water stress due to excess transpiration, by limiting the amount of functioning stomata. Greater area of leaf scorch leads to fewer functional stomata, which leads to less water loss per leaf, in this way trees with scorched leaves limit water loss by lowering their functioning evaporative leaf area.

Trees with scorched leaves had lower DBH than non-scorched trees. Height showed a positive correlation to DBH which is common for taller trees, to have a larger DBH than shorter trees they usually grow wider as well but there are factors impacting the height to diameter ratio (stem taper) such as windy environments (Larson, 1963). Tree height cannot directly be correlated with DBH the same in all environmental conditions, factors such as stand density in forests, or wind exposure of trees growing of hill tops can greatly affect DBH to height ratios (Telewski, 1995). However, a general trend can be established for this particular urban setting, which is, the taller trees had a larger DBH. The fact that a correlation between height and DBH showed such a strong relationship (p) = <0.00001 shows that my measurements were sound. Trees with greater height and larger DBH also had less soil compaction, so trees with less soil compaction grew faster.

Questions that arose from the preliminary work conducted in 2008 shaped the research conducted from 2009 until 2013. In 2008 photosynthesis efficiency measured as leaf fluorescence proved to be a valuable method of predicting which trees would soon exhibit scorch symptoms (Swartz et al. 2008). Later data collected as part of the present study in 2009 and 2010 did not show as strong a correlation between Photo system II and leaf visual scorch rating VSR. There was a similar effect between the Licor1600 data on leaf conductance. Both correlation matrixes showed no significant statistical correlations between VSR and either PSII (Table 5) or VSR and Cond (Table 6). Rather both PSII and Cond showed a highly significant statistical correlation between the data sets for 2009 and 2010, with Cond for 2009 and 2010. While PSII showed an almost identical trend for 2009 compared to 2010 data with negative correlation coefficient. VSR and Scholander data showed to same lack of correlation, but again showed a strong positive correlation between 2008 and 2009. The strong correlation between data sets shows a lack of operator error or erratic measurements, problems with the Licor 6400, the Licor 1600 or the Scholander Pressure chamber.

CONCLUSION

Iron rods that were extracted from the ground at sites BR1 BR2 and FR showed how diverse urban soils can be. During further examination of the rods I would often find area of anoxic soil in bands just above or below areas that showed high levels of soil oxygen. Further soil probes revealed that a layer, sometimes as thin as 10 cm existed above a compact soil that was composed of gravel, concrete and asphalt debris. In some places, this under layer was hard clay that could not be penetrated with a hand auger and less than a meter away there was soft sand more than a meter and a half deep.

Carnell & Anderson (1986) proposed a different method of interpretation of iron rods than that proposed by Hodge & Knott (1993) which I used to define the parameters of the iron rod experiment and data collection techniques. They recognized that there is a whole range of colors from brownish reds, to dull bright oranges to deep dark black with hues of blue. All these different colors can indicate not only if the soils were anaerobic, but also if they were water logged and at what time of year these occurred based upon the oxidization state of the iron. As I finished the research for this thesis, I came across the Carnell and Anderson paper, and realized that I had not given adequate attention to such details as the difference between orange-ish brown and dark brown, or black with hues of gray. The data I collected was cataloged as either 0 no rust, 1 mild rust, and 2 severe rust with no note of subtleties in color. The iron rods for our study were also allowed to stay in the soil for more than the three months recommended by both the paper by Carnell and Anderson as well as paper by Hodge and Knott. In fact, the iron rods were in the ground for almost six months, double the amount of time proposed by both papers by (Carnell & Anderson, 1986). Had I read this paper earlier perhaps the iron rod data would have shown a correlation, but in sites with as complex a soil profile as BR1, BR2 and FR perhaps there is no way to quickly and easily quantify oxygen availability in such varied soil strata.

APPENDIX B

SOIL AND FOLIAR NUTRIENTS

INTRODUCTION

Since leaf scorch is often a symptom of decline and mortality, my research has focused of leaf scorch as a means of quantifying tree health, by looking at differing levels of leaf scorch, and comparing scorch to planting practice, leaf nutrition, nutrient application, and soil nutrient availability that were collected over a six year period. This section will address the following questions: What are the possible correlations between leaf and soil nutrients and abiotic leaf scorch? If such correlations exist, can nutrient or pH imbalances be remedied? Lastly, is there a correlation between soil pH levels as they pertain to nutrient availability and abiotic leaf scorch?

Root systems require oxygen in order to respire. In heavy soils or soils with compaction issues the deeper the roots grow in the soil the less oxygen is available for them (Ohga & Ikushima, 1970; Weltecke & Gaertig, 2012). Planting a tree too deep, meaning that the soil surface is above the primary root flare, induces stress on the tree, often reducing survival, and growth rate (Bryan et al., 2006). The study of abiotic leaf scorch necessitates the examination of site factors for each scorched tree and comparison of the severity of site problems between neighboring non-scorched trees of similar species, age and site characteristics.

Interactions among the essential microelements also occur. Mn influences the transport of iron (Fe) in the leaf, leaves with Mn deficiency may contain higher than normal levels of Fe, furthermore foliar applications of Fe may decrease chlorosis symptoms in the high Fe-low Mn leaves (Messenger, 1983). Fe treatments are often used extensively with oaks but with little perceived benefit (Messenger, 1983). Mn oxidizes Fe converting it into an insoluble form preventing transport in from the leaf

(Chapman, 1931). As a catalyst for numerous enzymes and an important for structural element of the chlorophyll molecule Mn affects the efficiency of photosynthesis (Johnson et. al.,1988).

METHODS & MATERIALS

Study Sites:

As described in chapter 3:

Above Ground Parameters

Visual Scorch Rating:

As described in chapter 3:

Identification of Abiotic Stress:

As described in chapter 3:

Leaf Nutrient Analysis:

As described previously in Swartz et al. (2008), samples of leaves from trees exhibiting high levels of scorch and no scorch were collected from each site in July and separately analyzed for macronutrients and micronutrients at the MSU Soil and Plant testing Laboratory using the methods of (Walinga et al., 1995). Levels of deficiencies and toxicities for landscape trees were evaluated by comparing to tabulated reference data on red oaks by Walinga et al. (1995).

Mn. Injections/Soil Sulfur pH Experiments:

In 2009 a sub sample of trees at BR1, BR2, and FR were given injections of manganese using the *Mauget Inject-A-Min®* (Mauget Arcadia, CA USA). Manganese micro-nutrient formulation 6 ml pressurized capsules contain, 1% N, 1% K20, 1.9% S, 0.13% Cu, 1.67% Fe, 1% Mn, 0.12% Mg, 1.54% Zn. These preliminary experiments were performed to see if scorch symptoms could be alleviated in individual branches if exogenous Mn was injected at the branch bases (Figure 18), or in whole trees if injected at the base of the trunk (Figure 19). Another round of injections were applied in 2010 to trees at sites BR1, BR2, FR, SW, and GR. Finally, a third round of injections was performed on nine trees in a sub-plot experiment at sites BR1, and BR2 in March of 2011. The experiment consisted of three control trees, three trees which had micro-fine sulfur mixed with water applied to the area under their drip line, and three trees injected with the Mauget capsules (Figure 20). Each round of injections from 2009 - 2011 was performed with either photos taken to record any changes to the foliage, and or leaf tissue was collected for nutrient analysis. In some cases samples were collected from both before and after the injections for comparison of before and after treatments. Micro-fine sulfur application rates were calculated based on soil pH for each tree and the area under each trees drip line. Size of the area under the drip line was taken using an average of three measurements of the radius (R) from the trunk to the edge of the drip line:

$$\text{Area} = ((R) * (R)) * 3.14159 \qquad \text{Equation 1}$$

Assuming the soil to be mostly loam, sites BR1 and BR2 have an average pH of 8, so a conversion of 303 kilograms of sulfur per 4000 square meters or .007 kilograms per square meter. 7 kilograms per 1000 square meters was applied to make the appropriate pH adjustment from 8 to 6.5. Micro-fine sulfur was applied in a liquid form using GreenGarde® (GreenGarde, Bedford, PA. USA). Heavy-duty root feeder with a flow meter was used to inject the liquid directly into the soil (Figure 21) at a uniform depth of 25 cm. A 13.6 kilogram bag of Micro-fine sulfur was mixed with 378 liters of water in a pump trucks sprayer tank, thus 0.036 Kilograms per liter. For example, tree 91-06 at site BR2 had an average radius of 5 meters with a drip line area 79.46 square meters needed 6 kilograms of micro-fine sulfur applied in liquid form with 167 liters of water.

<u>Below Ground Parameters</u>

Soil Compaction (Pen (Joules/m)):

Soil compaction data was collected for sites BR1, BR2, FR, and AD, as previously reported in Swartz et al. (2008). The same method was used to collect data for sites SW, GR, and LB. Additional soil data was collected for sites GR, SW, and LB in May of 2013. These data were added to the existing data from the previous year's study for use in Pearson product moment correlation matrixes.

Soil Cores:

In 2009 soil sampling was done at sites BR1, and BR2. Transects were laid out across the two plots, with a core taken every ten feet across tree transects using an *AMS (*AMS, Inc. American Falls, ID USA) Basic Soil Sampling Kit purchased from Forestry Suppliers Inc. Cores were removed using a Sand Auger, 3 ¼ inch (8.3 cm) diameter, thread-on stainless steel Auger bucket, attached to a 4 foot (122 cm) thread-on extension, which was attached to an AMS thread-on cross handle. Coring was done to a depth of 91 cm, or until the corer reached an impediment. Cores where extracted, removed, placed in line next to the extraction hole, photographed, and three soil samples taken, one form the top, middle, and bottom of the core (Figure 15). Soil samples were analyzed to determine pH, and soil nutrient content (Mn, P, Mg, K, Ca and Al) was performed by the Soil and Plant testing Laboratory at Michigan State University using standard methods.

Statistical Analysis:

All statistical analyses were conducted using Sigmaplot version: 12 (Systat Software, Inc. San Jose, CA USA).

Soil pH and Sites Correlation:

An average of soil pH was analyzed using a one way analysis for variance, using pH as the dependent variable. Next a Shapiro-Wilk normality test was used to test for normal distribution. Next a Kruskal-Wallis one way analysis of variance on ranks was performed followed by a pairwise multiple comparisons of the sites using Dunn's method.

Mn. Injections/Soil Sulfur pH Experiments:

A Shapiro-Wilk's t-test for normality was run using Mn as the dependent variable against leaf samples taken before and after soil pH sulfur treatments or Mauget trunk injections were carried out. Next a Shapiro-Wilk's t-test for normality was run using Mn as the dependent variable against leaf samples taken from control trees. Finally, a Wilcoxon signed rank test was conducted using Mn as the dependent variable in comparison to control leaf samples. A Shapiro-Wilk's t-test for normality was run using Fe as the dependent variable against leaf samples taken before and after soil pH sulfur treatments or Mauget trunk injections were carried out. Next a Shapiro-Wilk's t-test for normality was run using Fe as the dependent variable against leaf samples collected from control trees.

Soil Nutrient Analysis:

A Pearson product moment correlation was run using visual scorch rating compared to soil nutrient level of Mn, P, Mg, K, Ca and Al.

Soil pH:

A Pearson product moment correlation was run using visual scorch rating compared to soil pH.

Leaf Nutrient Analysis:

A Pearson product moment correlation was run using visual scorch rating compared to leaf nutrient level of B, Ca, Fe, K, Mg, N, Cu and S.

RESULTS

Soil pH and Sites Correlation:

The average of soil pH was analyzed using a one way analysis for variance, using pH as the dependent variable. The analysis showed the median pH of sites BR1 and BR2 to be significantly higher ($p = < 0.05$) than site LB by 1.15, site GR by 0.4, and site SW by 0.3. The median pH values for the sites were BR1 & BR2 8, SW 7.7, GR 7.6, LB 6.4. There was no correlation between VSR and individual site pH.

Mn. Injections/Soil Sulfur pH Experiments:

Manganese: The data for Mn foliar concentration before and after soil pH sulfur treatments or Mauget trunk injections passed the Shapiro-Wilk's t-test for normality, (p) = 0.379 but showed no significance differences in either the two-tail (p) = 0.282 or the one-tail (p) = 0.141 tests. Leaf samples collected from trees that had been kept as controls failed the t-test for normality, (p) = > 0.050. Next a Wilcoxon signed rank test was conducted using Mn as the dependent variable in comparison to control leaf samples. A comparison of these medians showed a non-statistical significance (p) = 0.250. Lastly, the data from leaf samples taken from trees before and after they had been injected with Mauget Mn capsules, were tested using a Shapiro-Wilk's t-test for normality with Mn as the dependent variable against leaf sample data. These data passed the t-test for normality, (p) = 0.621 but showed no significance differences in either the two-tail (p) = 0.484 or the one-tail (p) = 0.242 analyses.

Iron: Using data collected from leaf samples, a Shapiro-Wilk's t-test for normality was run using Fe as the dependent variable against leaf samples taken before and after soil pH sulfur treatments or Mauget trunk injections were carried out. These data passed the t-test for normality, (p) = 0.197 and was highly significant in both the two-tail (p) = 0.00998 and the one-tail (p) = 0.00499. Similarly the trees that had been kept as controls for comparison had leaf samples taken before and after the other trees were treated. Using these data a Shapiro-Wilk's t-test for normality was run using Fe as the dependent variable against leaf samples taken at the same times as the other treatment trees were sampled. These data passed the t-test for normality, (p) = 0.570. A comparison of the before and after means showed no significant differences in either the two-tail (p) = 0.178 or the one-tail (p) = 0.0892. Lastly, the data from leaf samples taken from trees before and after they were injected with Mauget Mn capsules, where tested using a Shapiro-Wilk's t-test for normality using Fe as the dependent variable against leaf samples. These data passed the t-test for normality, (p) = 0.093, and showed no significance differences in the two-tail (p) = 0.0514 but did show a significant difference in the one-tail test (p) = 0.0257.

Soil Nutrient Analysis:

A Pearson product moment correlation was run using visual scorch rating VSR compared to soil nutrient level of Mn, P, Mg, K, Ca and Al. There was a non-significant relationship between any of the pair of variables in the correlation table with (p) = < 0.050 with Mn (r) = -0.211 (p) = 0.164, P (r) = -0.131 (p) = 0.390, Mg (r) = -0.00551 (p) = 0.971, K (r) = -0.243 (p) = 0.107, Ca (r) = -0.163 (p) = 0.286, Al (r) = 0.0822 (p) = 0.505.

Soil pH:

A Pearson product moment correlation was run using VSR compared to soil pH. No significant relationship was observed between any of the variables (r) = 0.267 and (p) = 0.767.

Leaf Nutrient Analysis:

A Pearson product moment correlation was run using VSR compared to leaf nutrient level of B, Ca, Fe, K, Mg, N, Cu and S. There was a non-significant relationship between any of the pair of variables in the correlation table with (p) = > 0.050 with B (r) = 0.122 (p) = 0.322, Ca (r) = 0.107 (p) = 0.385, Fe (r) = 0.177 (p) = 0.150, K (r) = 0.0295 (p) = 0.811, Mg (r) = 0.0281 (p) = 0.820, N (r) = 0.232 (p) = 0.0570, Cu (r) = 0.211 (p) = 0.0840 and S (r) = 0.206 (p) = 0.0919. The same test showed that VSR compared to Leaf nutrient levels of Mn, P and Zn did show statistically significant values. Mn compared to VSR showed a weak negative correlation coefficient of (*r*) = -0.356, yet showed a very strong statistical significance (*p*) = 0.00286. P compared to VSR showed a weak correlation coefficient of (*r*) = 0.287 with a highly statistically significant (*p*) = 0.0175. Zn showed a weak correlation coefficient of (*r*) = 0.239 and a statistical significance of (*p*) = 0.0497 (Table 9).

DISCUSSION

In order to attempt to address some of the possible correlations between VSR and nutrients, a series of experiments were conducted to see if I could impact leaf nutrient levels by altering the soil pH, or by directly injecting the nutrients that were perceived as deficient, namely Mn and Fe. Mn was determined to be lower in scorched leaves compared to non-scorched (Swartz et. al., 2008). A highly significantly negative correlation between Mn deficiency and leaf scorch was observed. The pressurized Mauget capsules that were used to inject Mn in to tree also contained Fe, N, K, S, Cu, Mn, Mg and Zn. Of these nutrients I focused on those that were known to be deficient (Table 10). Mauget injections did significantly increase Fe from a median amount of 60.53 ppm to 75.66 ppm, a difference of 15.13 ppm, and those trees that had micro-fine sulfur applied to their soil had their means for Fe increase from 77.58 ppm to 109.0 ppm with a highly significant difference of 31.42 ppm. The control trees that were also sampled before and after the treatments were carried out showed no increase in Fe, which shows that Fe nutrient deficiency can be mediated through soil pH with micro-fine sulfur application and direct trunk injection using Mauget capsules (Figure 22). However, the Mn levels in the leaf tissue samples did not show any change in either the Mauget injected trees, the trees that had pH adjusted with soil micro-fine sulfur or the control trees. This could mean that while the Fe levels within leaves increased in response to treatments, there was some other reason besides soil pH and availability that prevented the leaves from taking up the Mn (Table 10). Soil nutrient analysis showed Mn levels to be 60.77ppm, which is above the physiologically required level of 50 ppm (Table 10).

The nutrient data from the leaf samples collected from all other trees in the study showed a negative correlation between VSR and levels of Mn, also a positive correlation between VSR and both P and Zn (Table 11). A Mn concentration of 50 ppm in soil is considered adequate to support plant growth (Lucas & Davis, 1961). However, the availability of Mn is influenced by pH and a pH of 6.5 or less can create a deficiency (Lucas & Davis, 1961). Sites BR1 and BR2 had an average pH of 7.9 with site LB at the lowest levels with a pH of 6.8 (Table 12). All sites averaged together had a pH of 7.53 showing that all sites are either on the high side or very high side of pH as far as soil nutrient availability is concerned (Table 12). Therefore, pH is not impacting Mn availability to the roots of these trees. The trees in this study had an average of 17.45 ppm Mn in their leaf tissue samples; however their soil samples showed an average of 60.7 ppm of Mn, well above what is adequate (Lucas & Davis, 1961). Again there is more than adequate Mn available in the soil however, uptake is still being limited by a factor other than pH.

Another nutrient that showed a strong positive correlation with VSR was phosphorous, which showed an average of 0.17 ppm from leaf nutrient samples. P is considered to be in adequate levels at 0.2 ppm, (Lucas & Davis, 1961). Soil samples in this study averaged between 29 and 33.2 ppm, again well above what is required by the trees (Table 10). Early season foliage evaluations revealed that trees that had abiotic leaf scorch later in the growing season often would “Bronze” that is to turn a dark-reddish-purple, later becoming chlorotic and eventually scorching. This bronzing is a common sign of P deficiency, often with the bronzing progressing to necrotic spots (Marschner, 1995). Necrotic spots were also common in scorched trees. The isolated necrotic spots often expanded to become continuous brown interveinal areas that dried and became papery as the growing season progressed to late summer. P is also a mobile mineral element, (Marschner, 1995) it is possible that as the leaves begin to scorch the tree reallocates this important component of sugar phosphate, nucleic acids and phospholipids and ATP reactions, in order to conserve this mineral nutrient (Marschner, 1995).

Zn also showed a positive correlation to VSR, with an average leaf tissue sample of 26 ppm. Zn is considered to be at adequate levels at 20 ppm (Lucas & Davis, 1961) so once again, in this study, Zn is above what is considered adequate. Zn is important in enzyme activities, and chlorophyll biosynthesis in some plants. When deficient tree growth can be affected with reduced intermodal growth, leaves can develop a puckered appearance, and if sufficiently reduced plants are not ability to produce sufficient amounts of auxin indolic acid. Zn deficiency can cause interveinal chlorosis, and necrotic white spots on leaves (Marschner, 1995). Zn can be present in soil in forms unavailable to plants, there is a positive correlation between zinc in available forms and soil organic matter concentration " Zn2+ forms stable complexes with soil OM (organic matter)-components" (Tisdale et. al. 1993). Extensive soil cores and soil sample transects and the iron rod experiment (Appendix A) showed sites BR 1 and BR2 to have extremely disturbed compacted, hypoxic, and variable soils with a thin layer of topsoil to support turf. Under such soil conditions there would perhaps not be sufficient soil organic matter to support stable forms of zinc. Zinc is also linked to the formation of the enzyme alcohol dehydrogenase, which is key to anaerobic metabolism (Marschner, 1995). Soil pH data collected across all sites did not show a correlation to VSR (Figure 7) so it is not specifically the pH that is causing scorch, rather an interaction with nutrient availability and absorption that could be causing the scorch.

Under oxygen poor conditions such as can be found around root systems that are planted too deep or compacted soils, zinc deficiencies can lead to impaired metabolic function and root growth (Marschner, 1995). The mineral Mn, is important in enzyme activity, including carboxylase and dehydrogenase, though most important in the photosynthetic reaction that produces oxygen from water (Marschner, 1995). All three of these minerals manifest deficiency in the foliage, as either, bronzing, discoloration, necrotic spots, interveinal chlorosis, malformed leaves or some combination of all. It is possible that these are more than correlations between VSR and these nutrients, but may actually be physiological cause and effect.

CONCLUSION

Questions that arose from the preliminary work conducted in 2008 shaped the research conducted from 2009 to 2013. As it pertains to nutrient deficiencies, it is possible that the data collected show more than correlations between VSR and nutrients available in soil or interactions between pH availability limitations, but may suggest physiological cause and effect which require further elucidation. The complex interactions between, girdling roots, soil pH and nutrients that is available in the soil in different forms. For example, with Zn needing available organic matter in order to allow nutrient uptake in the form Zn^{2+} is one example of these interactions. Zn is shown to be in abundance in the soil. Perhaps the lack of oxygen as reveled by the iron rod experiments compounded with compaction by machinery during site construction can cause anaerobic conditions that can limit the breakdown of organic matter in the soil. Moreover, the fact that sites BR1 and BR2 are composed of the sub-soils from the deep excavation, some 8 stories beneath the surface of the earth, during the construction of the Breslin Center. However, although sub-soils are mineral rich, they don't have organic matter available to facilitate Zn uptake. This is but one example of the many ways that soil, trees, and nutrients interact. I examined more than 60 trees, across 7 sites looking at the way they interact with more than thirty different variables trying to find possible correlation and causes with VSR.

I have shown that trees with smaller DBH have higher scorch ratings, more compacted soils, and a greater percent of girdling roots. I have shown that trees that are planted too deep show greater leaf scorch, decreased DBH, and decreased height. I have shown that less compacted soils leads to taller trees with greater DBH, and those trees planted too deep show more scorch symptoms, which is correlated with nutrient levels of phosphorus, and zinc and deficiency in manganese. I choose site BR1 and BR2 for our nutrient experiments because it was the only site that had the required numbers of control and scorched trees but it also had a history of severe soil disturbance due to the construction of the Breslin center. Perhaps if a site with a less complex soil stratum was chosen, the results would be less convoluted, with few variables to consider when looking for possible causation as it pertains to VSR.

Site Irrigation and Data Collected by Year							
Year	AD	BR1	BR2	SW	FR	GR	LB
2008	X	X	X		X		
2009	X	X	X	X	X	X	
2010		X	X	X		X	
2011		X	X				
2012		X	X				X
2013							X
Irrigation Type							
Rain	X	X	X	X	X	X	X
Regulated Irr.				X			
Sporadic Irr.		X	X				

Table 1. Site Irrigation and Data Collected by Year:
Based on observations from previous years, established oaks exhibiting scorch adjacent to non-scorched oaks of the same age at each site, growing in the same soil types were chosen. Site (**AD**) = Oak hill Cemetery, Adrian, MI. (**BR1**) = Jack Breslin Student Events Center, MSU, MI. (**BR2**) = Jack Breslin Student Events Center, MSU, MI. (**SW**) = Shaw Lane, MSU, MI. (**FR**) = Farm Lane, MSU, MI. (**GR**) = Grand River Ave, East Lansing, MI. (**LB**) = Main Library, MSU, MI. Irrigation: (**Rain**) natural rain fall, (**Regulated Irr**.) systems controlled irrigation, (**Sporadic Irr.**) a member of the landscape staff manually turned on and off a sprinkler system, when it was deemed necessary absent of any schedule or known interval. (**X**) = year that data was collected at each site and the type of irrigation received from 2008 through 2013.

Cell Contents:
Correlation Coefficient
P Value
Number of Samples

	AGY	DORF	%GR	Pen (Joules/m)	VSR
BAGR (cm^2/yr)	0.0667 0.656 47	-0.348 0.0192 45	-0.217 0.157 44	0.469 0.000883 47	-0.252 0.0877 47
AGY		0.281 0.0619 45	0.0127 0.935 44	-0.0526 0.726 47	0.0847 0.571 47
DORF			0.329 0.0173 52	-0.0565 0.688 53	0.332 0.0151 53
%GR				-0.151 0.285 52	0.226 0.107 52
Pen (Joules/m)					-0.00106 0.994 60
VSR					

Table 2. Pearson’s product moment correlation table:
Correlation table comparing average basal area growth rate per year (BAGR), average growth per year (AGY), depth of original root flare (DORF) as measured from original soil surface to the top of the first tier, percent girdling root (%GR) is a measurement of the total percent of the root collar that is constricted by girdling roots compared to the total circumference of the base of the trunk, soil compaction (Pen (Joules/m)) or resistance of soil in the root zone quantified by determining soil resistance (impedance) to penetration and average visual scorch rating (VSR), rated as 0,1,2,or 3, for each tree at the seven sites,

Cell Contents:
Correlation Coefficient
P Value
Number of Samples

	Height (m)	DBH (cm)	Pen (Joules/m)	DORF (cm)	%GR
VSR	-0.131	-0.282	-0.00106	0.332	0.226
	0.319	0.0291	0.994	0.0151	0.107
	60	60	60	53	52
Height (m)		0.820	0.469	-0.0873	-0.201
		1.042E-015	0.000158	0.534	0.152
		60	60	53	52
DBH (cm)			0.454	-0.259	-0.120
			0.000269	0.0607	0.397
			60	53	52
Pen (Joules/m)				-0.0565	-0.151
				0.688	0.285
				53	52
DORF (cm)					0.329
					0.0173
					52
%GR					

Table 3. Original Pearson's product moment correlation table:
Correlation table comparing height (m), diameter at breast height (DBH), depth of original root flare (DORF) as measured from original soil surface to the top of the first tier, percent girdling root (%GR) is a measurement of the total percent of the root collar that is constricted by girdling roots compared to the total circumference of the base of the trunk, soil compaction (Pen (Joules/m)) or resistance of soil in the root zone quantified by determining soil resistance (impedance) to penetration and average visual scorch rating (VSR), rated as 0,1,2,or 3, for each tree at the seven sites. Due to the fact that the trees are not similar in age across sites it was important to change both height and DBH to correct for differences in age.

Cell Contents:
Correlation Coefficient
P Value
Number of Samples

	Leaf Temperature	Scholander LpP
VSR	0.634 0.00469 18	0.188 0.454 18
Leaf Temperature		-0.532 0.0230 18
Scholander LpP		

Table 4. Leaf temperature/Scholander (LpP) experiment correlation matrix:
Correlation matrix comparing visual scorch rating (VSR) to leaf temperature and Scholander pressure chamber (LpP) readings.

	Photo 2008	Photo 2009	Photo 2010
VSR	NS	NS	NS
Photo 2008		NS	NS
Photo 2009			***
Photo 2010			

* is p-value < 0.05; ** is p-value <0.01;
*** is p-value < 0.001

Table 5. Photosynthetic efficiency, PSII efficiency (Photo):
Pearson product moment correlation matrix was performed comparing photosynthetic efficiency data averages collected in 2008, 2009, 2010 (Photo 2008), (Photo 2009) and (Photo 2010) respectively, across all sites and VSR. There was a non-significant relationship between VSR and Photo in all years. Also there was a non-significant relationship when 2008 was compared to 2009 or 2010 photosynthetic efficiency data sets. There was a negative correlation coefficient (r) = -0.517 and an extremely strong significant similarity between the 2009 and 2010 photosynthetic efficiency data sets with (p) = 0.000235.

	Cond 2008	Cond 2009	Cond 2010
VSR	NS	NS	NS
Cond 2008		NS	NS
Cond 2009			***
Cond 2010			
* is *p*- value < 0.05; ** is *p* -value <0.01;			
*** is *p* -value < 0.001			

Table 6. Stomatal conductance, leaf gas exchange (Cond):
Pearson product moment correlation matrix was performed comparing stomatal conductance data averages collected in 2008, 2009, 2010 (Cond 2008), (Cond 2009) and (Cond 2010) respectively, across all sites and VSR. There was a non-significant relationship between VSR and Cond in all years. Also there was a non-significant relationship when Cond 2008 was compared to Cond 2009 or Cond 2010 data sets. There was an extremely strong correlation between the 2009 and 2010 stomatal conductance data sets with a positive correlation coefficient of (r) = 0.567 and an extremely strong significant similarity between the 2009 and 2010 stomatal conductance data sets with (p) = 0.0000496.

	Schol 2008	Schol 2009	Schol 2010
VSR	NS	NS	NS
Schol 2008		**	NS
Schol 2009			NS
Schol 2010			
* is *p*- value < 0.05; ** is *p* -value <0.01;			
*** is *p* -value < 0.001			

Table 7. Scholander pressure chamber, leaf pressure potential (Schol):
Pearson product moment correlation matrix was performed comparing Scholander pressure chamber data averages collected in 2008, 2009, 2010 (Schol 2008), (Schol 2009) and (Schol 2010) respectively, across all sites and VSR. There was a non-significant relationship between VSR and Schol in all years. Also there was a non-significant relationship between the Schol 2008 and Schol 2010 data sets, with similar results when Schol 2009 was compared to the Schol 2010 data. There was an extremely strong correlation between the 2008 and 2009 Scholander pressure chamber data sets with a positive correlation coefficient of (r) = 0.422 and strong significant similarity between the 2008 and 2009 Scholander pressure chamber data sets with (p) = 0.00924.

Cell Contents: Correlation Coefficient, P Value, Number of Samples	**Leaf Temperature**	**Scholander LpP**
VSR	0.634 0.00469 18	0.188 0.454 18
Leaf Temperature		-0.532 0.0230 18
Scholander LpP		

Table 8. Leaf temperature/Scholander experiment correlation matrix:
A correlation matrix was used to compare visual scorch rating (VSR), leaf temperature, and leaf pressure potential (LpP) averages.

Cell Contents: Correlation Coefficient, P Value, Number of Samples	**Zn**
Scorch	0.239 0.0497 68
Zn	

Table 9. Correlation table for zinc (Zn) and visual scorch rating (VSR):
Foliar nutrient (Zn) data collected from sites BR1 and BR2, compared to visual scorch rating.

	Mn (ppm)	P (%)	Mg (%)	K (%)	Ca (%)	Fe (ppm)	N (%)	Cu (ppm)	S (%)	B (ppm)	Zn (ppm)
VSR	** (neg)	* (pos)	NS	NS	NS	NS	NS	NS	NS	NS	* (pos)
Median	14.5	0.179	0.243	1.04	1.01	67	2.37	6.68	0.151	44	25
Adequate	50	0.2	0.2	1	0.5	100	1.5	6	0.1	20	20
Deficient Y/N	**Y**	**Y**	**N**	**N**	**N**	**Y**	**N**	**N**	**N**	**N**	**N**
* is *p*-value < 0.05; ** is *p*-value <0.01; *** is *p*-value < 0.001											

Table 10. Visual scorch rating (VSR) and leaf tissue nutrient analysis Pearson's correlations and foliar nutrient analysis levels compared to adequate levels looking for deficiencies:
Numerical results of correlation matrix of all leaf tissue analysis nutrients compared to visual scorch rating (VSR) TOP ROW. Lower rows show results of leaf tissue nutrient analysis, comparing Manganese (Mn), Phosphorus (P), Magnesium (Mg), Potassium (K), Calcium (Ca), Iron (Fe), Copper (Cu), Boron (B) and zinc (Zn) listed here is parts per million (ppm) along with Nitrogen (N) and Sulphur (S) as a percent (%), to known adequate nutrient levels, denoted as either deficient yes (Y) or not deficient no (N).

Cell Contents:
Correlation Coefficient
P Value
Number of Samples

	scorch
pH	0.267 0.0767 45
scorch	

Table 11. Correlation table for soil (pH) and visual scorch rating (VSR):
Soil pH data collected from sites BR1 and BR2, compared to visual scorch rating.

Soil pH Levels for Each Site			Soil Nutrient levels for all Sites		
BR	7.99			median	Ave
SW	7.73		Mn	61.8	60.77
GR	7.57		Mg	185	250.33
LB	6.83		Ca	2476	2575.27
All sites	7.53		K	71	90.09
			P	29.00	33.27

Table 12. Soil pH levels and soil nutrient levels:
Soil pH for sites BR1 and BR2 combined into (BR). Site FR is not included in this table, due to other treatments that were done to that site after preliminary work was completed.

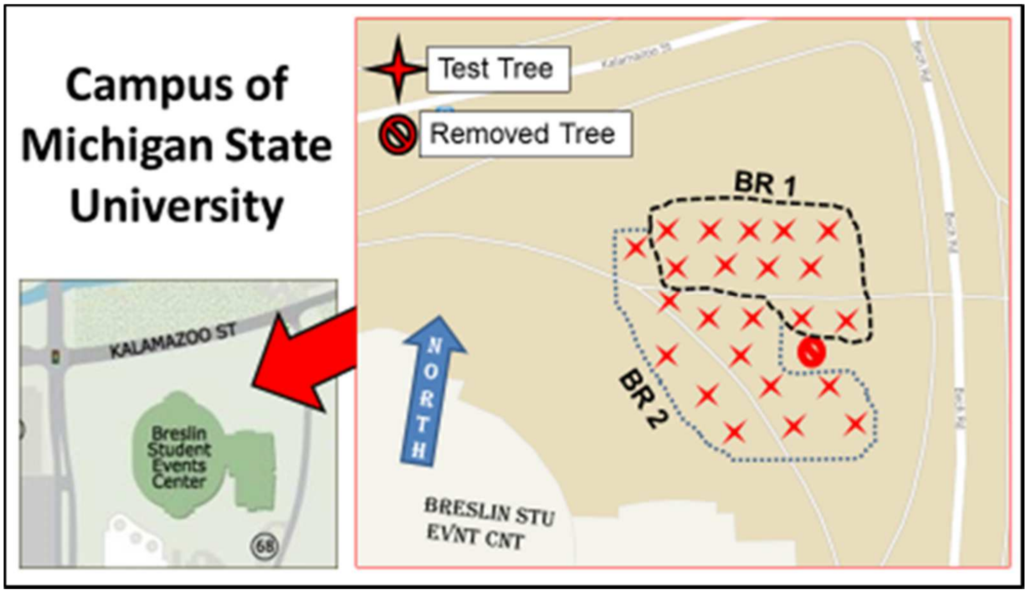


Figure 1. Sites BR1 and BR2:
Of the original three sites established in 2008, the first site, known as (BR), which stands for Breslin Center, also known as the Jack Breslin Student Events Center on the campus of Michigan State University, was split into two sites which are referred to as (BR1) and (BR2).

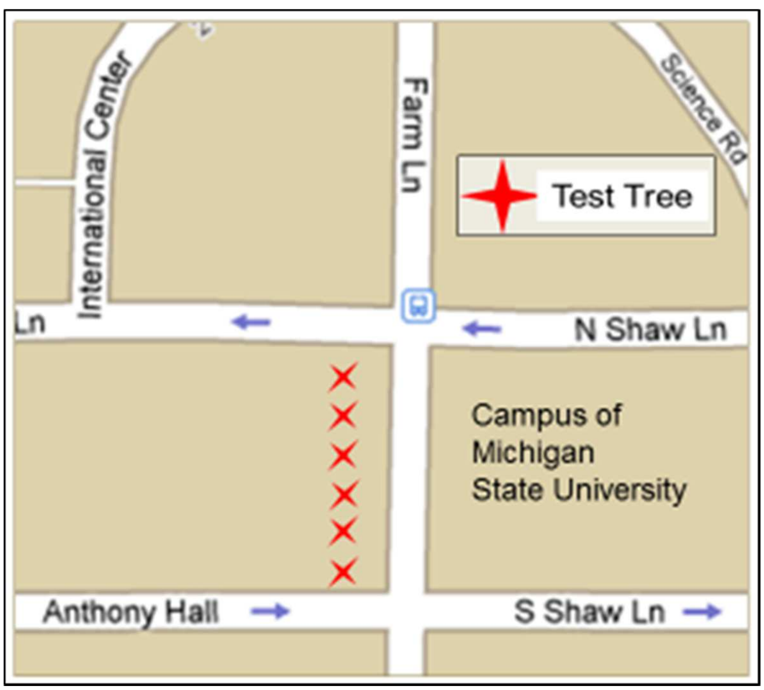


Figure 2. Site FR
The other original sites, Farm lane site on West side of Farm lane between East and West bound Shaw referred to as (FR).

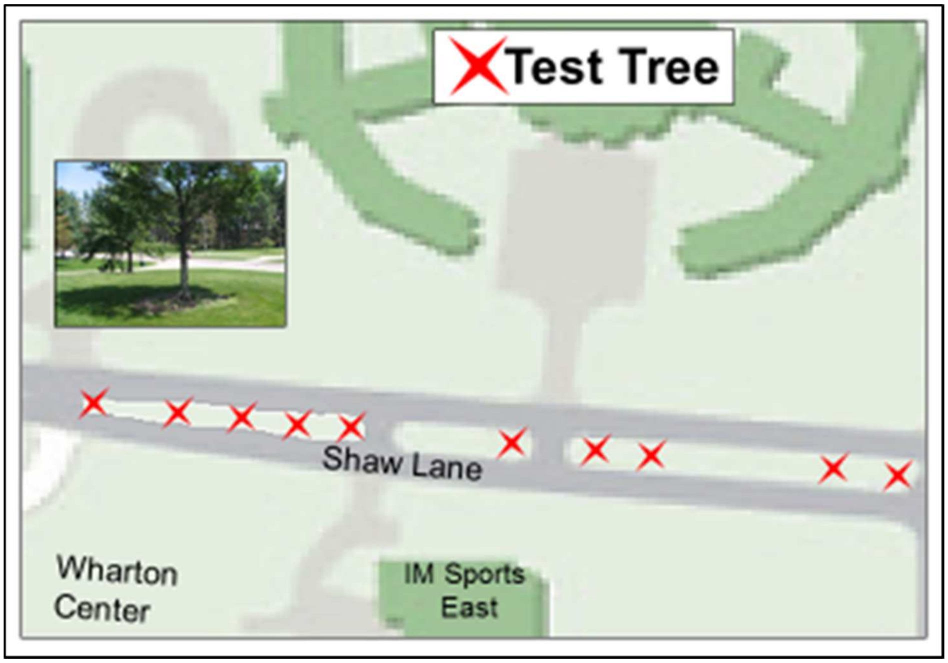


Figure 3. Site SW:
Two new sites were added in 2010. The first new site consisted of 10 trees along East Shaw lane between the Wharton Center and Wilson Road on the campus of Michigan State University. The Shaw lane site is referred to as (SW).

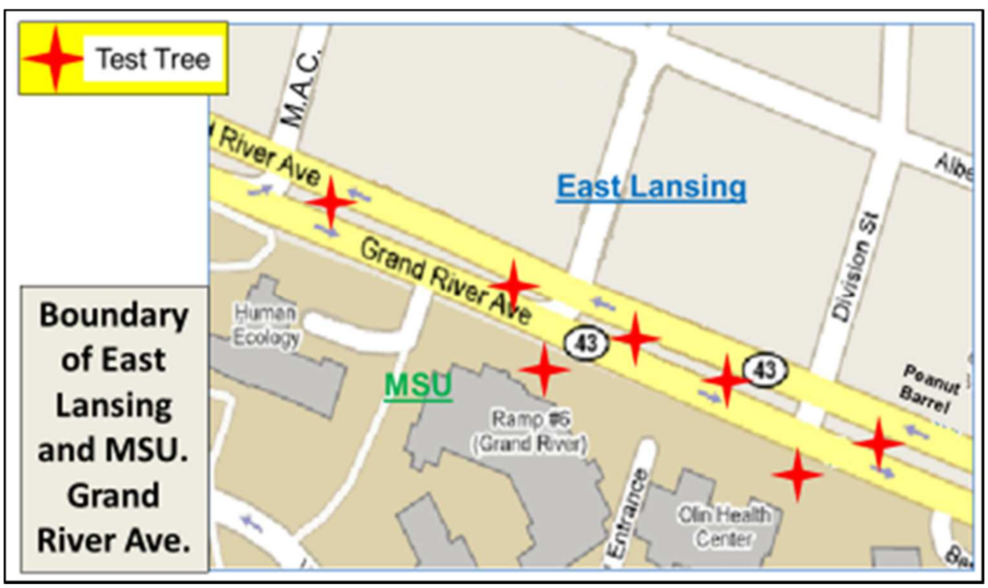


Figure 4. Site GR:
The second site established in 2010 was along Grand River Avenue, between M.A.C, and Bailey roads, referred to as (GR).

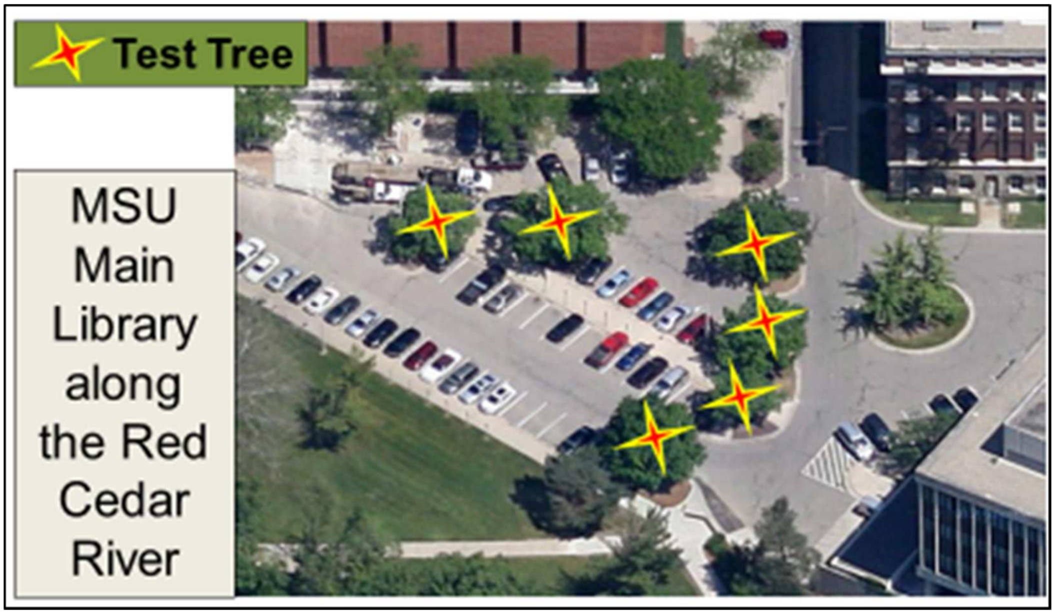


Figure 5. Site LB:
Finally in the fall of 2012, six more trees on the South side of the Michigan State University Main Library were added to the study referred to as (LB).

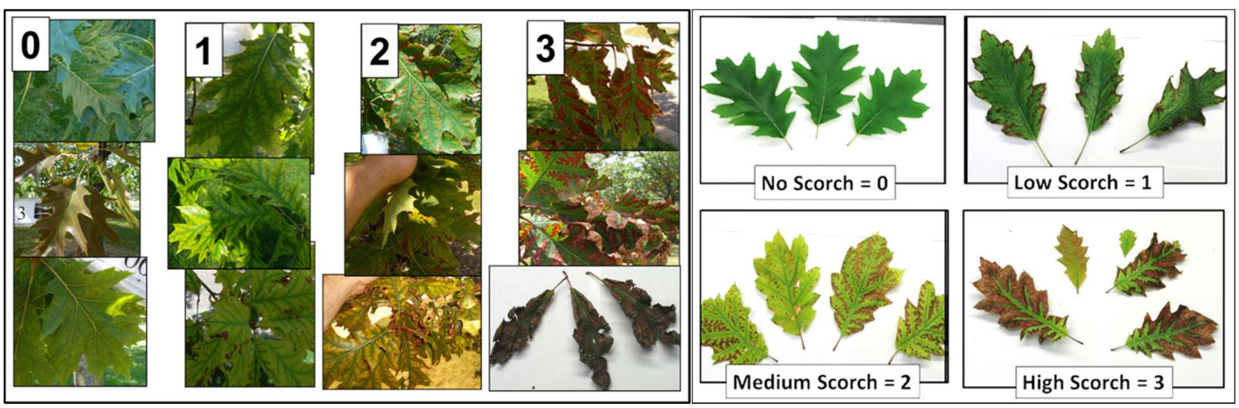


Figure 6. Visual scorch rating (VSR):
A scorch rating of 0 is a green leaf with characteristics of no discoloration to very mild interveinal discoloration (mild chlorosis). A scorch rating of 1 is a green leaf with symptoms ranging from medium chlorosis to mild small brown necrotic spots. A scorch rating of 2 is a leaf with severe chlorosis, and or some brown papery necrotic spots that have partially merged but do not cross the vein areas of the leaf. A scorch rating of 3 is a leaf that has symptoms ranging from having severe necrotic areas to being covered with brown dry papery necrotic areas in the interveinal areas, with some areas expanding over the leaf veins. The final scorch rating 3 is a leaf that dries to a point that it curls in an involute manner.

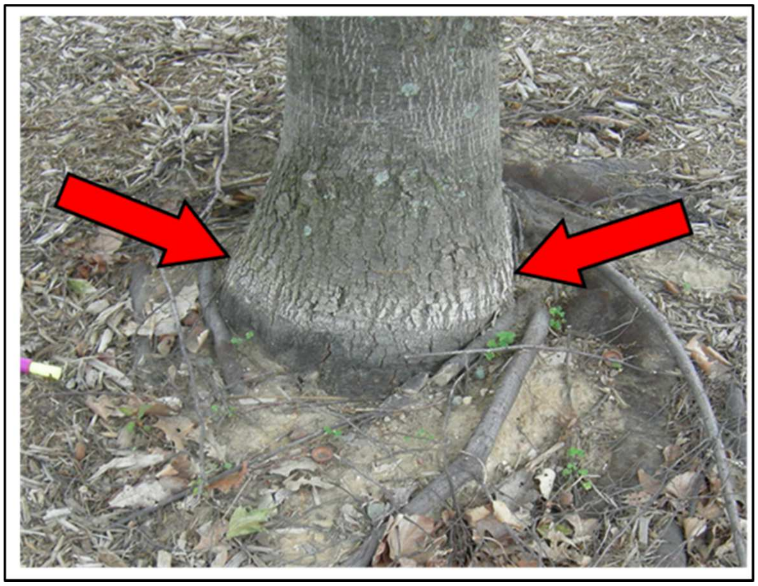


Figure 7. Photograph of mineral ring:
Photograph of tree 90-06 site FR. Girdling roots exposed after air spading was performed, removing several inches of soil and mulch. Months after the air-spading took place, a persistent mineral deposit ring could be observed on the tree trunk marking the mulch depth. This was used to determine the soil and mulch level for depth of original root flare (DORF) measurements.

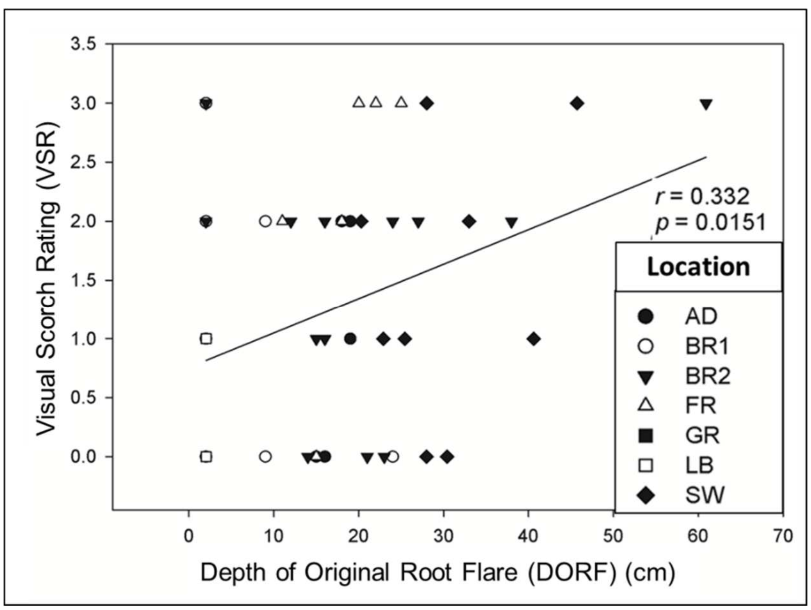


Figure 8. Correlation graph of VSR and DORF:
Average visual scorch rating (VSR), rated as 0,1,2,or 3, for each tree at the seven sites compared to the depth of original root flare (DORF) as measured from original soil surface to the top of the first tier, original root flare in centimeters. Location information see table 1.

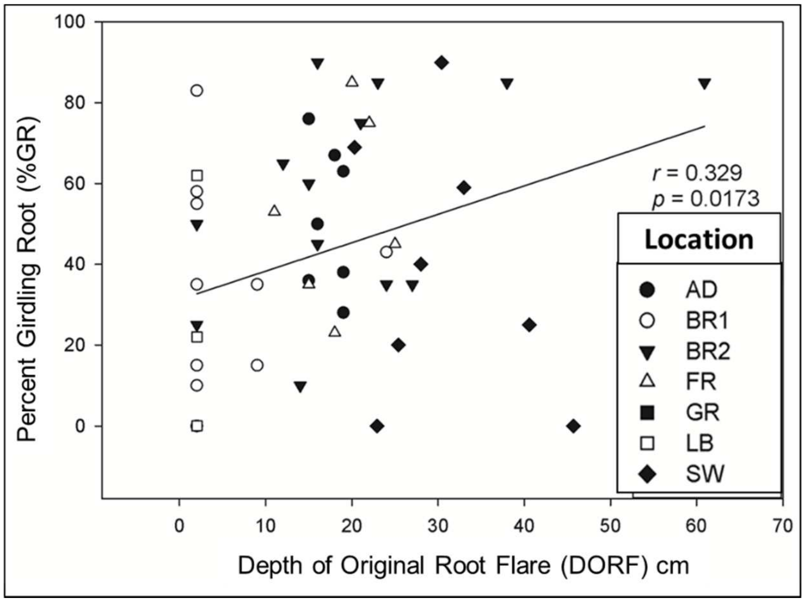


Figure 9. Correlation graph of %GR and DORF:
Percent girdling root (%GR) is a measurement of the total percent of the root collar that is constricted by girdling roots compared to the total circumference of the base of the trunk compared to the depth of original root flare (DORF) as measured from original soil surface to the top of the first tier, original root flare in centimeters. Location information is listed in Table 1.

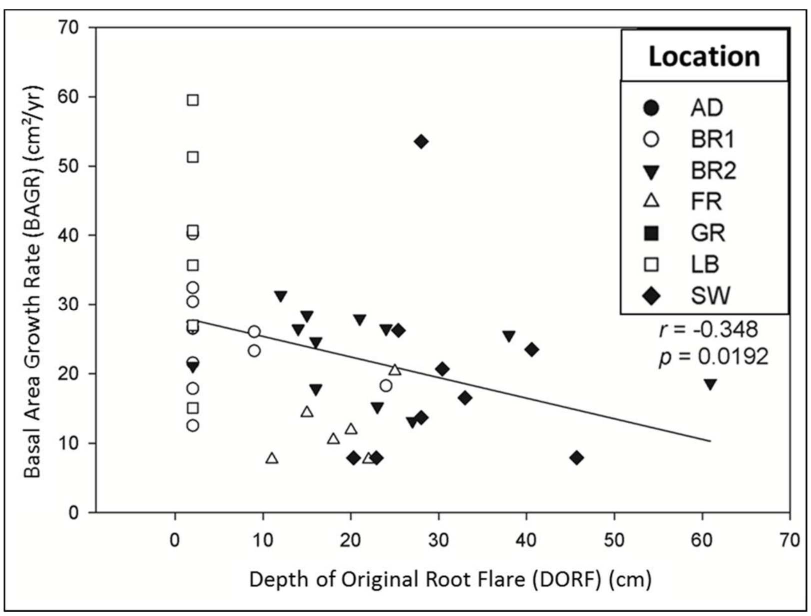


Figure 10. Correlation graph of BAGR and DORF:
Basal area growth rate per year (BAGR) measures the average increase in basal area of the trunk per year of growth. Cross sectional area of the trunk in cm^2, along with the determined tree age, was used to calculate average cross sectional growth rate per year. This was compared to the depth of original root flare (DORF) as measured from original soil surface to the top of the first tier, original root flare in centimeters. Location information is listed in Table 1.

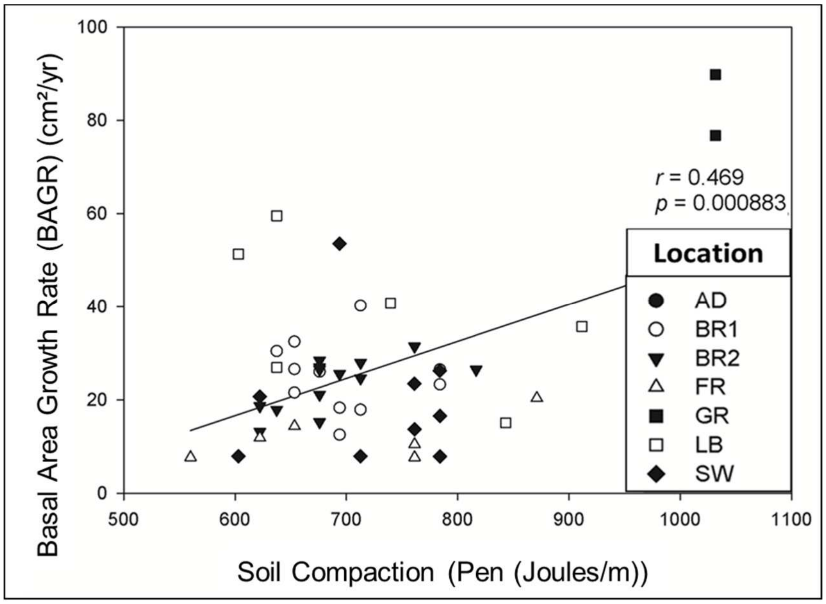


Figure 11. Correlation graph of BAGR and DORF:
Basal area growth rate per year (BAGR) measures the average increase in basal area of the trunk per year of growth. Cross sectional area of the trunk in cm^2, along with the determined tree age, was used to calculate average cross sectional growth rate per year. This was compared to the depth of original root flare (DORF) as measured from original soil surface to the top of the first tier, original root flare in centimeters. Location information is listed in Table 1.

Figure 12. Measuring depth of original root flare (DORF) at site AD:
Demonstration of the technique used to measuring depth of original root flare.

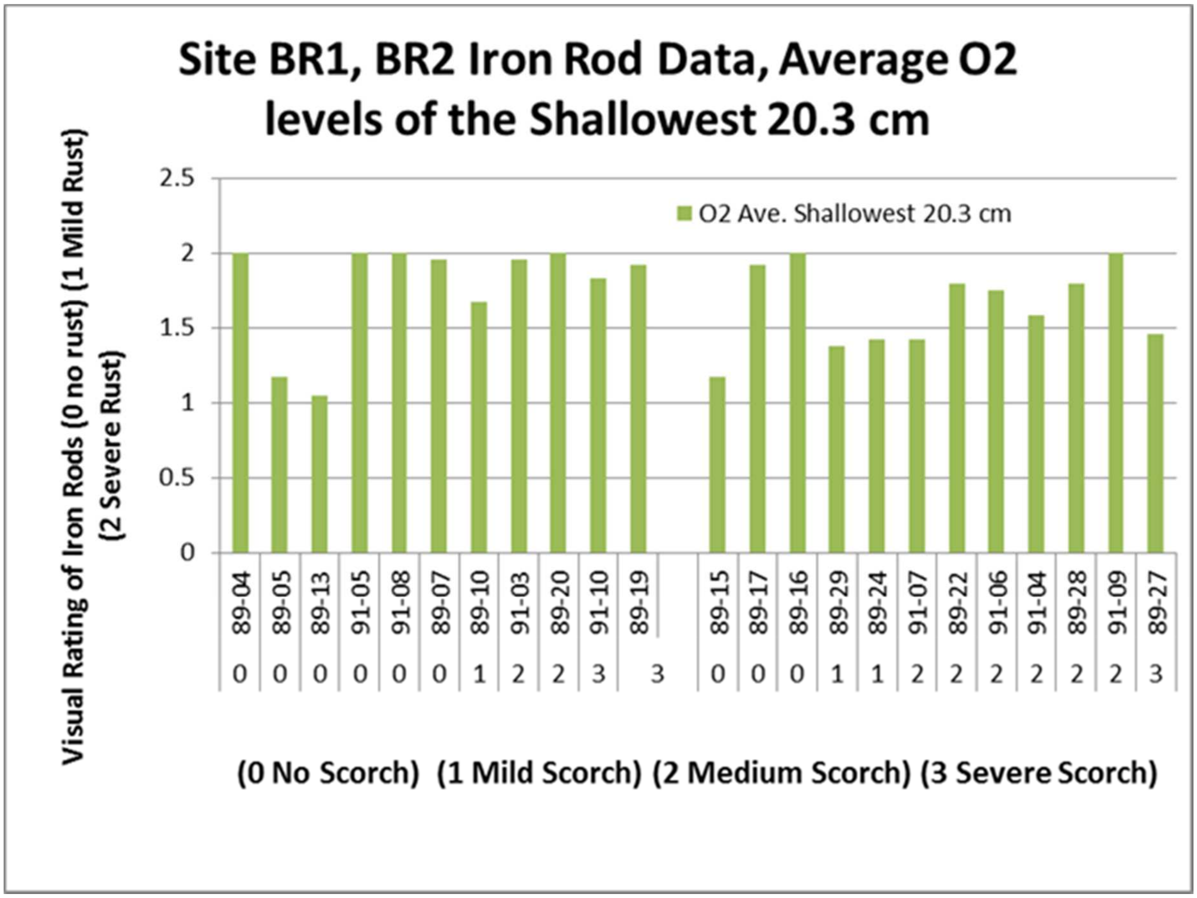


Figure 13. Iron rod data for BR1 and BR2:
Soil oxygen levels and variations in soil strata were measured by driving iron rods into the ground, and later retrieval for visual inspection (Hodge & Knott, 1993). Three iron rods each made of high carbon steel from Alro steel (Alro Corp. Jackson, MI USA), 0.5 cm wide and 60 cm long, were driven into the ground around each of the test trees at each site in one of the four cardinal directions North, South, East, or West as determined by a compass, in random order. One rod was placed between 30 and 40 cm of the tree, the second at the half the distance from the trunk to the drip line of the tree, and the third at the drip line. Sites BR1 and BR2 had Iron rods installed between July 22nd and 28th 2008. The rods were left in the ground over the winter and removed the following spring starting on March 20th 2009. Each of the iron rods was located using maps and a metal detector. Rods where removed, cleaned with a soft bristle brush wiped with a shop towel, placed on a white back ground with an identification tag, and photographed for later visual inspection. Each of the rods was examined and a rust rating of no rust (0), mild rust (1) or severe rust (2) was assigned for every 2 cm of each rod.

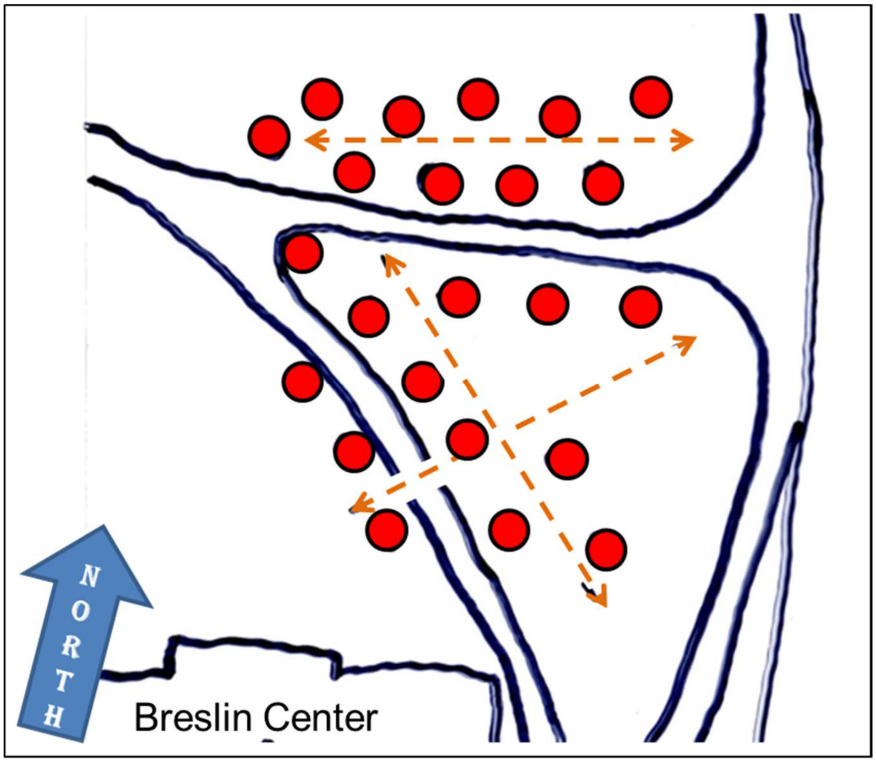


Figure 14. Soil core transects BR1 and BR2:
In 2009 soil sampling was done at sites BR1, and BR2. Transects were laid out across the two plots, with a core taken every ten feet across three transects.

Figure 15. Soil core auger:
Picture of an AMS (AMS, Inc. American Falls, ID USA) Basic Soil Sampling Kit, cores were removed using a Sand Auger, 8.3 cm diameter, thread-on stainless steel Auger bucket, attached to a 122 cm thread-on extension, which was attached to an AMS thread-on cross handle. Coring was done to a depth of 91 cm, or until the corer reached an impediment that could not be cleared. Cores where extracted, removed, placed in line next to the extraction hole, photographed, and three soil samples taken, one form the top, middle, and bottom of the core.

Figure 16. Cores to determine top soil depth:
Further soil cores were done in May 2013 to collect measurements of the depth of topsoil layer at sites BR1 and BR2 for comparison to Iron rod data from the shallowest 20cm. Using the original map of iron rod placement, each tree at the two BR sites had three cores taken with a 21 inch AMS® soil probe, in as close an approximation to the placement that the original iron rods were inserted in the ground. Soil samples were extracted, visually assessed for soil texture, and depth of top soil layer was measured in centimeters with a ruler. In this way a field average was collected for later comparison.

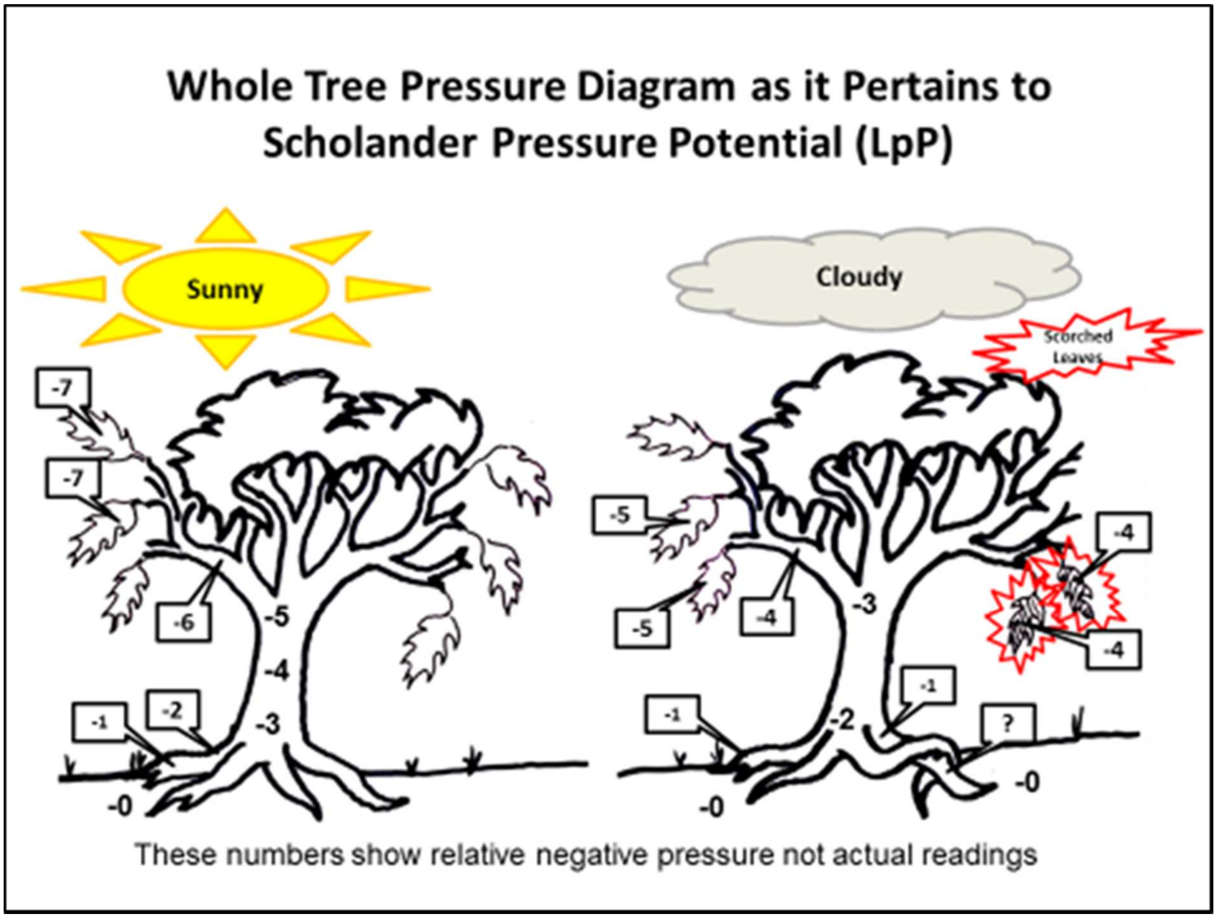


Figure 17. Leaf temperature/Scholander experiment:
In 2012, a series of three tests were done at sites BR1 and BR2, comparing scorch and none scorch tree, and the possible relationship between leaf temperature and leaf pressure potential. Leaf temperature data was collected using an Extech® Instruments, (Extech® Instruments Nashua, NH U.S.A) InfaRed thermometer model 4252, IR gun (+or- 2% of reading), from ten different leaves in each of the six test trees. Of the six trees, three had a history of scorch, and three were non-scorched trees. Information on ambient temperature was collected by hanging a mercury thermometer in the under canopy of a nearby control tree, allowing it to acclimate for a minimum of 15 minutes, and taking a measurement before and after site work was initiated, along with cloud cover conditions. In addition, leaves were also collected from each of the test trees. For this experiment the leaves were not transported to the lab to obtain stomatal conductance data, rather the Scholander pressure chamber, Model 600, (PMS Instrument Company Albany, OR. USA) and the nitrogen tank were transported to the BR test site so that Scholander readings could be taken in the field.

Figure 18. Mauget branch injections:
In 2009 a sub sample of trees at BR1, BR2, and FR were given injections of Manganese using the *Mauget Inject-A-Min*® (Mauget Arcadia, CA USA). Manganese micro-nutrient formulation 6 ml pressurized capsules containing, 1%N, 1%K20, 1.9% S, 0.13% Cu, 1.67% Fe, 1%Mn, 0.12% Mg, 1.54% Zn. These preliminary experiments were performed to see if scorch symptoms could be relieved in individual branches if injected at their bases.

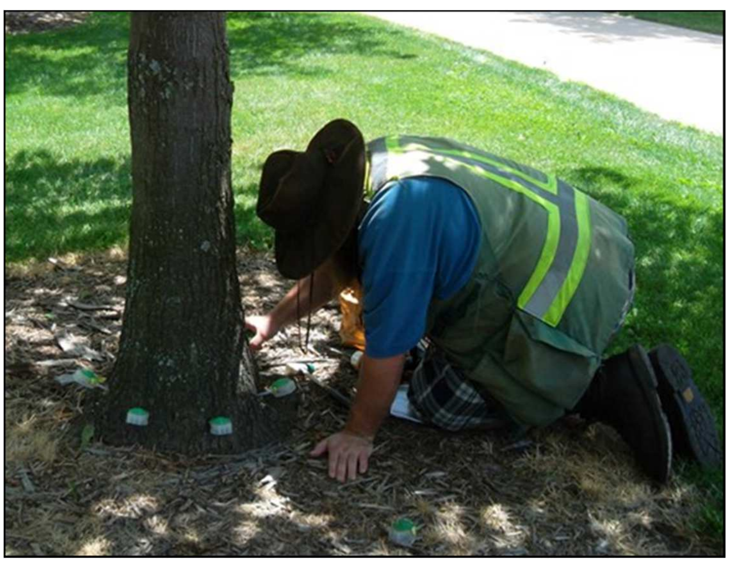


Figure 19. Mauget trunk injections:
There was another round of injections in 2010 to trees at sites BR1, BR2, FR, SW, and GR.

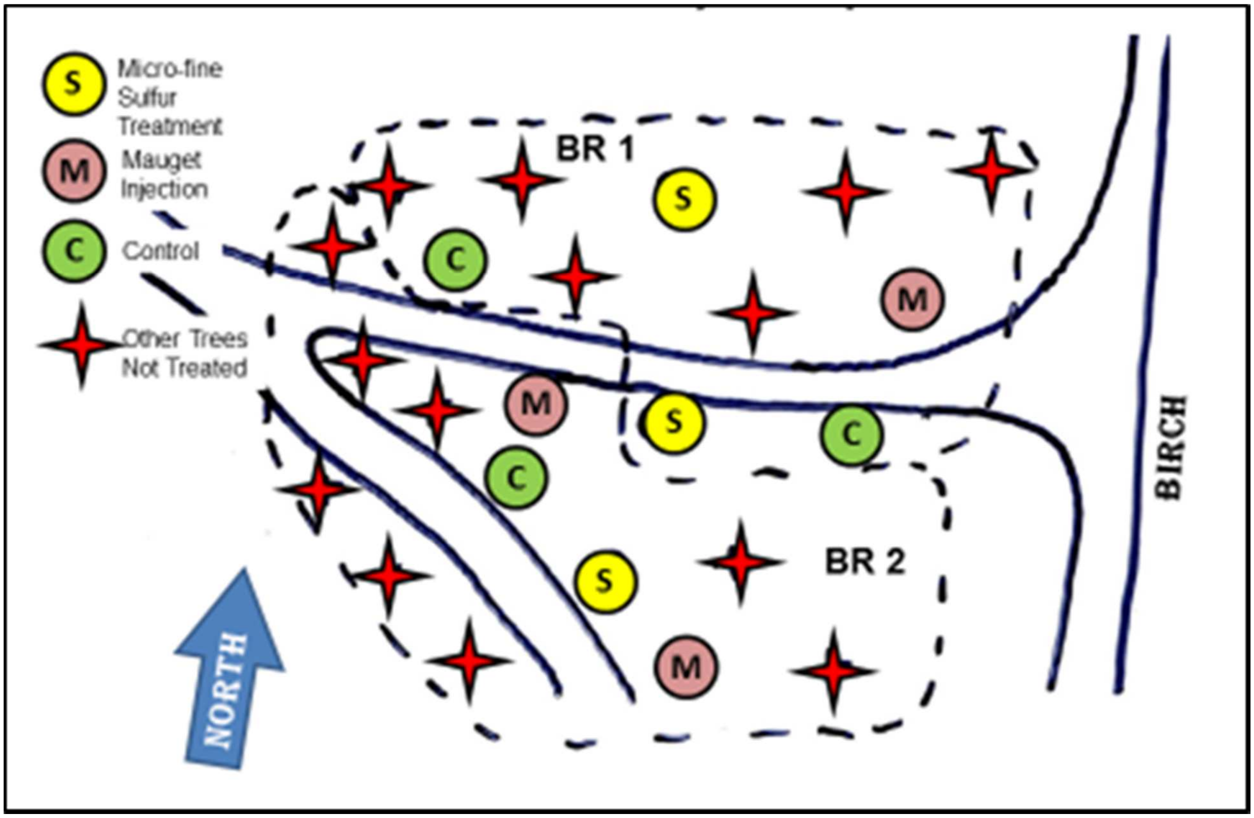
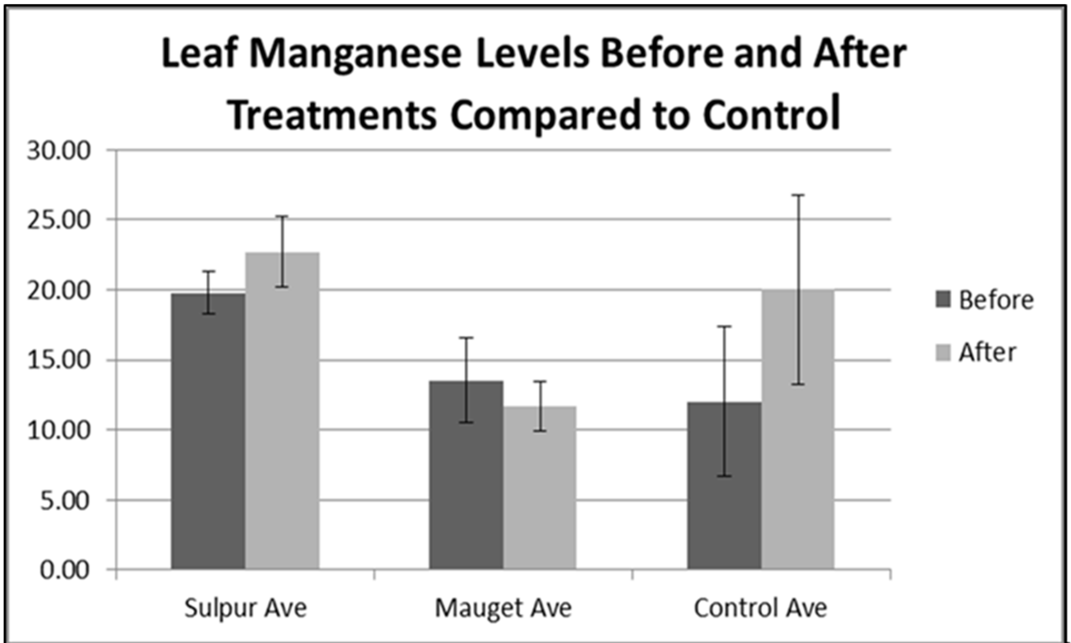


Figure 20. Mn. injections/soil sulfur pH experiments:
Finally, a third round of injections was performed on nine trees in a sub-plot experiment at sites BR1, and BR2 in March of 2011. The experiment consisted of three control trees (Marked here as green circles with "C", three trees which had micro-fine sulfur mixed with water applied to the area under their drip line (Marked here as yellow circles with "S", and three trees injected with the Mauget capsules (Marked here as pink circles with "M". Each round of injections from 2009 - 2011 was performed with either photos taken of any changes to the foliage, and or leaf tissue was collected for nutrient analysis. In some cases both were done before and after the injections for comparison. Micro-fine sulfur application rates were calculated based on soil pH for each tree and the area under each trees drip line.

Figure 21. Soil sulphur injection:
Micro-fine sulfur was applied in a liquid form using GreenGarde® (GreenGarde, Bedford, PA. USA). Heavy-duty root feeder with a flow meter was used to inject the liquid directly into the soil at a uniform depth of 25 cm.



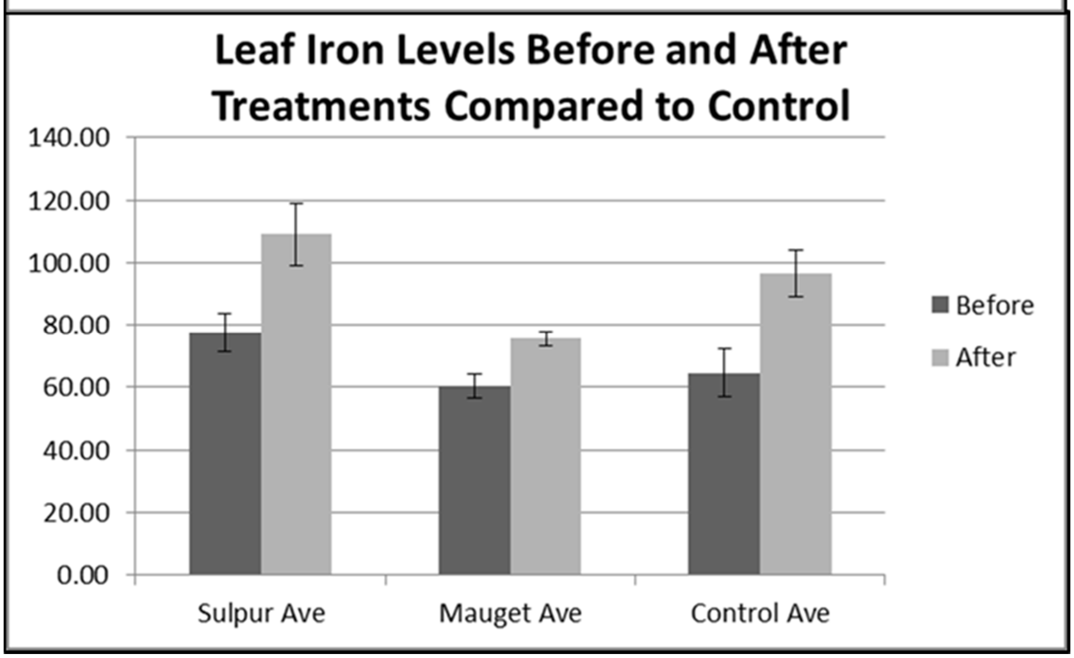


Figure 22. Mn. injections/soil sulfur pH experiments:
Using data collected from leaf samples, Mn and Fe levels in leaf samples taken before and after soil pH/sulfur treatments or Mauget trunk injections were carried out. Similarly the trees that had been kept as controls for comparison had leaf samples taken before and after the other trees were treated.

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LITERATURE CITED

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