EFFECTS OF CARBOHYDRATE APPLICATIONS ON GROWTH AND VITALITY

OF LIVE OAK (*Quercus virginiana*)

A Dissertation

by

TOMÁS MARTÍNEZ TRINIDAD

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

May 2008

Major Subject: Forestry

EFFECTS OF CARBOHYDRATE APPLICATIONS ON GROWTH AND VITALITY

OF LIVE OAK (*Quercus virginiana*)

A Dissertation

by

TOMÁS MARTÍNEZ TRINIDAD

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Approved by:

Chair of Committee,	W. Todd Watson
Committee Members,	Michael A. Arnold
	David N. Appel
	Leonardo Lombardini
Head of Department,	Steven Whisenant

May 2008

Major Subject: Forestry

ABSTRACT

Effects of Carbohydrate Applications on Growth and Vitality of Live Oak (*Quercus*

virginiana). (May 2008)

Tomás Martínez Trinidad, B.S., Universidad Autónoma Chapingo, México;

M.S., Colegio de Postgraduados, México

Chair of Advisory Committee: Dr. W. Todd Watson

Urban forests grow in stressful environments that can have negative repercussions on tree energy reserves. The goal of this research was to evaluate the impact of exogenously applied carbohydrates on growth and vitality of live oaks (*Quercus virginiana* P. Miller). An initial study focused on carbohydrate partitioning revealed that annual mean glucose concentration in leaf tissues (49.55 mg·g^{-1} DW) was almost double that in twigs, trunks, or roots. Starch concentrations in roots and trunks (38.98 and 38.22 mg·g^{-1} DW of glucose, respectively) were higher during the dormant season and approximately three times the concentrations found in other tissues. An investigation of the effects of exogenous soil applications of glucose and starch on soil microbial activity revealed no significant differences using recoverable viable microbes. However, soil respiration was significantly increased ($P<0.05$) by glucose a week after application, while higher starch concentrations (120 g·L^{-1}) significantly increased ($P<0.05$) soil respiration after the fourth week. Although tree soil drenched with carbohydrates in a different study showed significantly ($P<0.05$) greener leaf color,

higher chlorophyll fluorescence, and increased soil respiration at higher concentrations of starch (120 g·L^{-1}), no significant differences were observed in photosynthesis or trunk, canopy, or root growth. Analysis of δ^{13}C signatures was unable to detect uptake of exogenous carbohydrates. For trunk-injected trees with glucose and sucrose, trunk growth was significantly ($P<0.05$) increased by carbohydrate supplementation. Differences were also found in twig glucose content, root starch content, and chlorophyll fluorescence among overall concentration means. A study to compare field diagnostic tools with carbohydrate laboratory analysis established that a portable blood glucose meter can be used to measure glucose content in trees. However, ohmmeter, refractometer, chlorophyll fluorescence spectrometer, and iodine staining results did not correlate well with laboratory analysis of carbohydrate concentrations. Results from these studies reveal that soil applied carbohydrates can greatly increase soil microbial activity, provide evidence that trunk-injected carbohydrates may improve growth and vitality of live oaks, and provide a new field diagnostic tool to increase the efficiency of measuring carbohydrates in trees.

ACKNOWLEDGEMENTS

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER I

INTRODUCTION

Trees growing in urban environments typically experience numerous stressful conditions that have negative effects on energy reserves. Many of the stressors can be reduced by altering site conditions and using species adapted to the site; however, trees still have to survive the adverse conditions present in urban areas. Most of the negative conditions, such as pollution, soil contamination, impervious areas, soil compaction, heat islands, restricted growing areas, and diseases, may reduce photosynthesis, the physiological process of carbohydrate production in trees (Taiz and Zeiger, 2006). A lack of carbohydrate production and the depletion of stored carbohydrates can cause tree decline or death (Kosola et al., 2001; Wargo et al., 1972).

Source of Energy in Trees

Carbohydrates are the principal products of photosynthesis reactions which consist of reduction of atmospheric CO_2 by the use of light and the release of oxygen from water (Taiz and Zeiger, 2006). Photosynthesis can be described through the generalized equation:

$$6CO_2 + 6H_2O \xrightarrow{\text{light}} C_6H_{12}O_6 + 6O_2$$
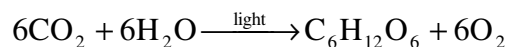
Some of the first carbohydrates produced by photosynthesis in woody plants are glucose and fructose which are the most common simple sugars, also called

_____

This dissertation follows the style of HortScience.

monosaccharides, (Pallardy, 2008). Simple carbohydrates synthesized by the photosynthetic process are converted later into storage forms of energy. Sucrose, a disaccharide composed of two simple sugars (glucose and fructose), is the main carbohydrate used to translocate sugars to other plant parts through the vascular tissue (Taiz and Zeiger, 2006). The other important carbohydrate is starch, a polysaccharide. Starch is considered the major carbohydrate reserve in woody plants (Pallardy, 2008). Starch has been shown to be more important as a reserve food than lipid or simple sugars in *Quercus alba* L. (McLaughlin et al., 1980).

The concentrations of different carbohydrates can vary depending on environmental conditions. Some research indicated that the concentration of starch was independent of the soluble sugar concentration in xylem (Gregory and Wargo, 1985). Research also indicated that concentrations of starch and simple sugar decreased as a response to stressful conditions (Kosola et al., 2001; Tainter and Lewis, 1982; Tschaplinski and Blake, 1994). Under stressful conditions, such as defoliation and drought, starch levels declined while reducing sugars, glucose and fructose, increased in *Quercus velutina* Lam. (Parker and Patton, 1975). An increase of the levels of reducing sugars has also been reported as a result of cold temperatures (Levitt, 1980; Nguyen et al., 1990).

Carbohydrates can be used as source of energy by trees for different processes such as reproduction, defense, maintenance, storage, or growth (Lilly, 2001). The reduction of starch levels represents complex physiological changes rather than a simple change in carbohydrate metabolism (Wargo et al., 1972). Many carbohydrates are

continually undergoing conversion from one form to another (Pallardy, 2008), or they can be used for generating energy and transformation into other compounds through respiration (Nelson and Cox, 2005). The respiration reaction can be generally expressed in this equation:

$$C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O + \text{energy}$$
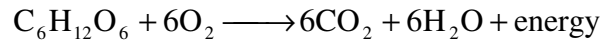
The most common use of starch reserves is for maintaining respiration and growth when carbohydrates are not supplied directly from photosynthesis (Pallardy, 2008). Carbohydrate reserves influence the capacity in trees to support growth, metabolism, and even survival under stressful conditions (Kaelke and Dawson, 2005).

Carbohydrate Partitioning in Trees

The translocation of carbohydrates within a tree is ruled by source-sink relations that are affected by environmental conditions or development stages (Allen et al., 2005; Grulke et al., 2001; Retzlaff et al., 2001). Carbohydrates can be translocated from sources (organs that export photosynthates) to sinks (organs that import photosynthates) (Tschaplinski and Blake, 1994). Mature leaves are the main sources of carbohydrates, and they export sugars to other parts of the plant, while roots store high concentration of sugars mainly as starch (Pallardy, 2008). Mature leaves can contribute to the elaboration of the new leaves or reconstitution of the starch reserves by the production of photosynthetic sugars rather than the mobilization of their starch reserves (Alaoui-Sosse et al., 1994).

Trees vary in the allocation or use of carbohydrates stored in tissues. In deciduous trees, roots and trunk serve as the main storage organs during the dormant

season and typically will be depleted shortly before leaves begin to emerge, while evergreen trees seem to store considerable amounts of starch in leaves and branches (Grulke et al., 2001; Larcher, 1980; Newell et al., 2002; Retzlaff et al., 2001). Deciduous trees require extensive carbohydrate storage to maintain the living biomass and cope with stress-inducing factors (Abod and Webster, 1991; Gansert and Sprick, 1998). For example, *Q. alba* rapidly mobilizes and replaces starch reserves during the critical period of canopy generation in the spring (McLaughlin et al., 1980). Conifers accumulate carbohydrates in needles and twigs prior to bud-break and translocate them during the beginning of shoot growth (Ludovici et al., 2002). *Pinus sylvestris* L. allocates high percentages of sugar in needles as a response to low soil temperatures (Domisch et al., 2002). Under urban conditions, carbohydrate allocation can be affected when tree organs are modified by human activities such as root pruning (storage), canopy pruning (sources) or trunk damage (vascular system) (Harris et al., 2004).

Carbohydrate reserves help to offset low carbohydrate production due to stressful conditions or high demand. Differences in the allocation of carbohydrates to storage tissues could arise from differential requirements of different organs, different needs during growth, or maintenance respiration required among different species (Barbaroux et al., 2003; Dean, 2001). Differences in starch concentrations could indicate different rates of production, demand, or shifts in allocation (Ludovici et al., 2002). In trees, the continuous pathways of transport and storage of previous-year assimilates are essential for subsequent growth processes, so the coordination and interrelations of morphogenic and photosynthetic processes are very important (Kaipiainen and Sofronova, 2003).

Carbohydrates can be used under stress conditions as a precursor for secondary compounds used to resist stress (Renaud and Mauffette, 1991; Webb, 1981). Understanding carbohydrate partitioning in trees can help in understanding the relationships between phenological stages and movement and utilization of available energy resources (Pallardy, 2008) as well as provide a reference for tree vitality.

Tree Vitality

There has been some confusion concerning the use of the terms vitality and vigor. In this paper, vitality will be referred as the plant's ability (health) to deal effectively with stress, while vigor is the genetic capacity to grow and resist stress (Lilly, 2001). The importance of various carbohydrates in the vitality of trees has been studied (Abod and Webster, 1991; Gregory and Wargo, 1985; Tainter and Lewis, 1982). Most of these researchers have focused on starch, glucose, and sucrose levels, the primary, stored and translocated carbohydrates in trees (Alaoui-Sosse et al., 1994). The results indicated the importance of sufficient carbohydrate levels, mainly starch, as a way to improve tree vitality (Carroll et al., 1983; Wargo et al., 2002). When trees are affected by stressful conditions, carbohydrate level can decrease or become depleted, which can have negative repercussions on growth and vitality (Gregory and Wargo, 1985).

Although previous research pointed out the importance of carbohydrate levels in relation to tree vitality, there is little research about the exogenous application of carbohydrates to trees. Arboricultural practitioners have recommended applications of several products such as growth regulators, fertilizers, mycorrhizal fungi, and recently small quantities of sugar to improve plant vitality (Harris et al., 2004: Percival et al.,

2004; Percival and Smiley, 2002). The determination of the effects of sugar applications on tree vitality may provide valuable and practical data to assist arborists in rehabilitating declining trees.

Effect of Carbohydrates as Soil Amendment

A common method of applying products to trees is through root drenches. Soil properties should be considered when assessing the applicability of applying carbohydrates under field conditions. Research about soil-applied carbohydrate uptake in crop plants showed that root cells were able to uptake carbohydrates from the soil (Stanzel et al., 1988; Stubbs et al., 2004). It is well reported that plants supply soil microorganisms with carbon as an energy source by exudates from root tissues (Ros et al., 2003; Schmidt et al., 1997b). Previous research suggested that adding sucrose to the root zones of seedling trees could improve root growth (Percival et al., 2004). When applying sugars to the root system under field conditions, the role of soil microbes should be considered because microorganisms can use carbohydrates before they are absorbed by roots (Jonasson et al., 1996).

Soil microorganisms are an important component in the use of root drenches with carbohydrate solutions. The effect of carbohydrates on microorganisms depends on the type of sugar, amount of leaching, potential root uptake, and microbe degradation or sequestration (Wagner and Wolf, 2005). Small amounts of carbohydrates can be quickly used by microorganisms (Schmidt et al., 2000) or be lost due to other factors, such as leaching, before they can have an effect on trees. Applications of carbohydrates can increase soil microbial activity considering that soil usually contains low amount of

carbohydrates (Illeris and Jonasson, 1999). Michelsen et al. (1999) indicated that microbial activity can affect the rate of nutrient uptake by tree roots making nutrient elements more available to plants. However, Jonasson et al. (1996) also pointed out that an increase in microbial populations can temporally immobilize nutrients in the soil and affect plant growth. A soil property that can be affected by carbohydrate amendments is the carbon to nitrogen (C:N) ratio, which can affect the rate that the amendment is consumed by microorganisms (Bloem et al., 1997).

Organic amendments can improve the physicochemical and biological properties of soil and thus their productiveness and natural fertility (Pascual et al., 1997). Mineralization of carbohydrates in soil may depend on the density of microorganisms involved and the dependency on other microbial transformations (Dassonville et al., 2004). Microbial activity in soils can be used as an indicator of soil quality; therefore, it can also be used to assess the effectiveness of soil treatments (Chidthaisong and Conrad, 2000; Ilstedt et al., 2000). Considering the potential effects of carbohydrates on improving site quality and tree vitality, research about the effects of sugar amendments on soil quality and subsequent tree growth and vitality needs to be conducted on established, field-grown trees.

Methods for Assessing Microbial Activity in Soils

Soil respiration and microbe plate counting methods have been commonly used to determine microbial activity in soils. Both techniques can be adapted to determine the effectiveness of soil treatments or cultural practices (Chidthaisong and Conrad, 2000; Ilstedt et al., 2000). The dilution plate count technique consists of collecting soil

samples, preparing serial dilutions, and plating dilutions on media to estimate microbial populations (Alexander, 2005; Parkinson, 1982; Zuberer, 1994).

The first concern in enumerating soil microbes results from their population density in soils; for example, bacteria, actinomycetes, and fungi population densities can vary from 10^8 to 10^9, 10^7 to 10^9, and 10^5 to 10^6 propagules per g dry soil respectively (Tate, 2000). In addition, some soil microbes may be killed in the dilution process or fail to grow on the plating media. Therefore, plate counting methods only estimate a small part (about 1%) of total microbial populations, which are able to growth under laboratory conditions. However, this method is still considered useful for contrasting soil microorganism populations of soils under differing treatments (Alexander, 2005; Zuberer, 1994).

Soil respiration is considered to be the most common nonspecific measurement of microbial activity (Tate, 2000). Soil respiration can be measured either in the field or the laboratory using different methodologies based on the type of information needed. Titrimetric analysis of CO_2 trapped in alkali solvents, known also as the alkali trap method, remains a popular and frequently used method because of its simplicity and high degree of sensitivity (Anderson, 1982). This technique can be easily adapted to the experimental conditions. For example, the technique can consist of placing a soil sample into a container with a smaller container of sodium hydroxide (NaOH) over the soil. The container is tightly sealed to avoid gas leakage and incubated for a known period of time. After incubation, phenolphthalein and $BaCl_2$ are used to precipitate the carbonates. Samples are titrated with HCl, and CO_2 evolution is estimated based on the amount of

HCl required during the titration (pink color disappears). The results are contrasted from the controls, and the value of CO_2 emitted is estimated from the value for the soil samples (Anderson, 1982; Pascual et al., 1997). Previous research indicates the high relationship between CO_2 emitted by soil respiration and microbial activity (Bååth and Arnebrant, 1994; Ros et al., 2003; Tate, 2000).

Exogenous Carbohydrate Uptake Assessed by $\delta^{13}C$ Signatures

Atmospheric CO_2 contains the two stable isotopes of carbon atoms, ^{13}C and ^{12}C, and is composed of approximately 1.1% ^{13}C and 98.9% ^{12}C (Farquhar et al., 1989). Even when the chemical properties of both isotopes are identical, plants discriminate against the heavier isotope of carbon (^{13}C) during photosynthesis, thus presenting smaller $^{13}C/^{12}C$ ratios than that present in atmospheric CO_2 (Taiz and Zeiger, 2006). The natural abundance of heavy stable isotopes (^{13}C) relative to the respective lighter isotope (^{12}C) in a given sample is traditionally expressed by the numerical ratio of atoms of heavy to light isotope (Pate and Dawson, 1999). The carbon isotope ratio ($\delta^{13}C$) of plants is expressed on a per mill basis (‰) (Taiz and Zeiger, 2006):

$$\delta^{13}C‰ = \left(\frac{R_{sample}}{R_{standard}} - 1 \right) \times 1000$$

where the standard represents the carbon isotopes contained in a fossil belemnite from the Pee Dee limestone formation of South California (Peterson and Fry, 1987).

C$_3$ plants discriminate against $^{13}CO_2$ to a greater extent than do C$_4$ plants (Taiz and Zeiger, 2006). For example, $\delta^{13}C$ values for C$_3$ plants range from -20 to -34‰ while $\delta^{13}C$ values for C$_4$ plants range from -9 to 16.8‰ (Pate and Dawson, 1999). Carbon

isotope ratio ($\delta^{13}C$) reflects the time-integrated ratio of internal to ambient CO_2 concentrations which is a function of stomatal conductance and photosynthesis capacity (Taiz and Zeiger, 2006). Therefore, $\delta^{13}C$ signatures can be used to differentiate between C_3 and C_4 photosynthesis and can also be used to obtain information about stomatal conditions for plants grown in different conditions. For example, $\delta^{13}C$ signatures have been used for screening population adaptation to dry sites (Garcia et al., 2004) as a tool for assessing plant responses to climate change (Fotelli et al., 2003), and recently in ecological studies to determine the C_3 and C_4 composition of ecosystems using plant tissues or soil organic matter (Eleki et al., 2005; Sinton et al., 2000; Stock et al., 2004). Considering that carbon isotope ratio ($\delta^{13}C$) from plant tissues has been used to differentiate between C_3 and C_4 plants, analyzing $\delta^{13}C$ signatures can help to determine the uptake of exogenous sugar when solutions containing sugars from C_4 plants such as corn or cane are applied to the root systems of C_3 plants such as live oaks.

Trunk Injections for Supplementing Carbohydrates

Arboricultural practitioners inject fertilizers, pesticides and other compounds into trees to improve tree vitality. The most common injection methods include bark banding, trunk infusion, and pressurized trunk injections (Sachs et al., 1977; Sanchez and Fernandez, 2004). Trunk injections can be classified as micro- or macroinjections based on the diameter (< or > 3/8") and depth (< or > 1") of the injection wound (Costonis, 1981). Microinjections use smaller amounts of higher concentrated solutions that macroinjection techniques. They can also be grouped as high or low pressure injections (> or < 100 kPa, respectively) (Sanchez and Fernandez, 2004). Macroinfusion is a

macroinjection method that has been successfully used in the application of considerable amounts of solutions into trunks of trees (Appel, 2001; Eggers et al., 2005). This technique uses modified garden sprayers, plastic tubing, and connector and infusion ports (tees) that allow the injection of high volumes with a pressure between 104 to 138 kPa (Appel, 2001). Research has shown that pressurized injected solutions in the trunk can move upward and downward through the vascular system (Sachs et al., 1977; Tattar and Tattar, 1999).

As with soil-applied carbohydrates, there is very little research on trunk-injected carbohydrates. Most of these studies have been conducted in crops and fruit trees and have shown little effect (Abdin et al., 1998; Iglesias et al., 2003; Zhou et al., 1997). The results might have been affected by the type of plant or the applied carbohydrate concentrations used in previous research since the injection system used in the research delivered low volumes. Trunk injections are targeted to deliver substances directly into the vascular system, mainly the xylem (Harris et al., 2004). According to Sanchez and Fernandez (2000), under urban conditions this technique offers the following advantages: more efficient product use, elimination of environmental contamination, a viable alternative to ineffective or costly leaf or ground treatments, and an alternative where other methods prove to be problematic.

One of the disadvantages of using trunk injection is the creation of wounds in the tree trunk; consequently, small wounds are recommended to lower the potential for wood decay in trees. Another disadvantage of using some low pressure and microinjection systems is the small and variable amount of solution that enters the tree

(Iglesias et al., 2001). Sanchez and Fernandez (2000) indicated that the factors that can influence the uptake and distribution of substances injected can be the type of substance injected, the injection site, the specie treated, the tree transpiration rate, the stress condition, the wind speed, the soil water content, the tree size, the tree vitality, and the phenological stage. In addition, frequent injections are not recommended because wounds can increase the risk of future decay in the tree (Costonis, 1981). Due to the potential risk caused when using trunk injections, there is some research that does not recommend the use of trunk injections (Perry et al., 1991). However, trunk injection methods have shown to be effective in the applications of different products such as hormones, fungicides, micronutrients, and sugars without reporting any considerable future damage caused by wounds (Costinis, 1981; Iglesias et al., 2001; Mayhead, 1991; Osterbauer and French, 1992; Percival and Boyle, 2005; Worley and Littrell, 1981). The applications of carbohydrates through trunk injections can be an option to increase the carbohydrate content in the system, which can have an effect on growth and vitality (Abdin et al., 1998; Giedraitis, 1990; Iglesias et al., 2001).

Methods for Assessing Tree Vitality

Environmental factors can have negative or positive effects on tree vitality; therefore, finding a way to measure and improve tree vitality has been an important but difficult task for arborists. As mentioned before, tree vitality has been described as a tree's ability to deal with stress (tree health). In many cases, tree vitality has been evaluated in terms of growth (Dobbertin, 2005). Other studies have described vitality by measuring carbohydrate levels, mainly the starch content on roots (Wargo, 1975; 1976).

Variables related to photosynthesis efficiency or cambium vitality have also been used in assessing this variable (Percival and Fraser, 2001; Shigo and Shortle, 1985).

Tree growth is the most common indicator used for studying the effect of environmental factors or treatments; therefore, this variable has been used for assessing vitality (Polak et al., 2006). The problem with using this variable relies on the fact that multiple measurements are required and arborists usually do not have access to the tree growth history. Tree height, trunk diameter, or root growth have all been used as indicators of tree vitality (Dobbertin, 2005). Trunk diameter usually is the easiest variable to measure. However, growth by itself may not necessarily indicate tree vitality because trees with high growth rates can also be susceptible to stressful conditions (Harris et al., 2004).

Carbohydrate content in different tree organs has been considered as another way to assess vitality in trees (Carroll et al., 1983; Kosola et al., 2001; McCullough and Wagner, 1987; Wargo et al., 1972). The relationship between carbohydrate content and vitality is based on the role of carbohydrates as the main source of energy in trees. Carbohydrate reserves in most deciduous trees reflect the photosynthesis capacity of the plant (Pallardy, 2008). The content of carbohydrates in tree tissues indicates the translocation activity as well as the storing capacity of energy in trees (Bardaroux et al., 2003).

The most accurate method for determining carbohydrate content in tree tissues is done through laboratory analysis (Haissing and Dickson, 1979; Wargo, 1975). However, field techniques, such as the starch profile using the Lugol's solution, have been

suggested in the determination of carbohydrate content (Dobbertin, 2005; Wargo et al., 2002; Wargo, 1979). The refractometer has also been used for measuring glucose content in fruits or vegetable tissues (Waes et al., 1998). Both tools have been suggested as an easy, practical, field technique in the determination of starch or glucose in different types of samples (Waes et al., 1998; Wargo, 1979). The use of digital meters for the determination of glucose can be also adapted in the estimation of glucose from tree tissues. In any case, carbohydrate content must represent the overall condition of the tree species to be a reliable and useful index (Carroll et al., 1983; Renaud and Mauffette, 1991; Wargo, 1976).

New tools have been suggested in the determination of tree vitality. One such tool is a chlorophyll fluorescence spectrometer. Among the chlorophyll parameters, Fv/Fm ratio measures the quantum efficiency of the photosystem II (Maxwell and Johnson, 2000) and has been suggested in the determination of tree stress and tree vitality (Percival and Sheriffs, 2002). Several research studies have shown how this variable has been used in the assessment of tree vitality (Percival and Fraser, 2005; Percival, 2004; Percival and Sheriffs, 2002; Percival and Fraser, 2001). The availability of portable chlorophyll fluorescence spectrometers provides easy, rapid and useful measurements under field conditions (Percival and Fraser, 2001).

Electrical resistances in the cambial zone in the tree trunks have also been suggested as an easy technique for assessing tree vitality (Blanchard et al., 1983; McCullough and Wagner, 1987). Resistance readings indicate the moisture and ion content of the cambial zone (Shigo and Shortle, 1985), which may indicate the size of

the cambial zone. Research indicates that electrical resistance at the trunk provides information about physiological changes and vitality of trees (Blanchard et al., 1983; Filip et al., 2002; Weston et al., 1979). Field ohmmeters, such as the Shigometer®, are suggested as a practical tool to assess electrical resistances, and thereby vitality, in trees (Dunn and Rowland, 1986; Ostrosfsky and Shortle, 1989; Paysen et al., 2006; Wargo et al., 2002). However, there is research indicating that this tool did not provide acceptable results in *Liquidambar stryraciflua* L. (Clark et al., 1992).

CHAPTER II

TEMPORAL AND SPATIAL CARBOHYDRATE PARTITIONING IN LIVE OAK

Carbohydrates are the principal products of photosynthetic activity and the main energy reserve of trees (Tromp, 1983). Some of these products are used by organs where they are produced (e.g., leaves) or are translocated to other organs, a phenomenon controlled by sink-source relations (Allen et al., 2005). Therefore, carbohydrates can be translocated from sources (organs that produce photosynthates) to sinks (organs that produce little or no carbohydrates) where they can be utilized or stored (Taiz and Zeiger, 2006). These sink-source relationships are influenced by tree vitality, nutritional state, environmental conditions, and the developmental stage of plants or tissues (Grulke et al., 2001; Retzlaff et al., 2001; Tschaplinski and Blake, 1994). Understanding carbohydrate activity is critical in stressful environments, such as urban forests, where tree health is negatively impacted and environmental stressors are frequent.

Research highlights the important role of carbohydrate reserves on a tree's ability to tolerate stressful conditions (Bardaroux et al., 2003). Nonstructural carbohydrates (i.e., starch and soluble sugars) influence the capacity in trees for supporting growth, metabolism, and ultimately their survival (Kaelke and Dawson, 2005). Unfortunately, most of the research information about reserve translocation in trees is usually described in young plants grown in greenhouses or natural environments rather than in older trees where carbohydrate production, translocation, utilization, and storage may differ greatly (Domisch et al., 2002; Gansert and Sprick, 1998; Tognetti and Johnson, 1999;

Tschaplinski and Blake, 1994). In addition, much of the recent research concerning carbohydrate partitioning has focused on a single season or short period of time (Barbaroux et al*.,* 2003; DeLucia et al*.,* 1998; Retzlatt et al*.,* 2001).

Trees in urban environments are subjected to numerous environmental stressors throughout the year that negatively impact carbohydrate production, utilization, and/or storage. Live oak, *Quercus virginiana*, is a common species found in urban environments in the southern United States due to the species' adaptability to poor sites, low maintenance requirements, disease resistance, and long life span, which make this species suitable as an ornamental for urban environments (Gilman and Watson, 1994; Little, 1979). Although live oaks are deciduous, their leaves continue to function throughout the winter until the trees defoliate in the spring during budbreak. Understanding carbohydrate partitioning in live oaks will be valuable for future research studies investigating the effects of exogenous applications of carbohydrates or regulation of photosynthate production, translocation, utilization, or storage to improve the health and survivability of urban trees.

The objectives of this research were to study the carbohydrate content, glucose and starch, in tree roots, trunks, twigs, and leaves of large, field-grown live oaks as well as to determine the impacts of seasonal influences on carbohydrate concentrations.

Materials and Methods

Plant material

Five, field-grown live oaks, approximately 10-cm caliper measured 30 cm above ground, were randomly selected within a nursery at Monaville, TX (29º57'1.59"N,

96º3'28.73"W). Trees selected were planted on 5-m spacing and grown under similar conditions.

Tissue samples from roots, trunk, and canopy were collected from opposing sides of the tree (along and between rows of trees) corresponding to the four cardinal points (north, south, east, and west). Root samples consisted of 4-mm diameter increment cores from the buttress (woody) roots (2 cm from the base of the trunk). Tissue from the trunk, 4 mm in diameter, was collected using an increment hammer (Haglof©; Langsele, Sweden) at 1.3-m height from the ground. Increment cores were approximately 100 mm in length. Canopy samples consisted of five leaves and twigs, which were randomly collected from the lower two-thirds of the canopy (Mclaughlin et al., 1980). Samples from the different parts of the trees were collected in July 2005, September 2005, January 2006, and March 2006 (Fig. 2.1).

Samples were stored on blue ice (Rubbermaid®, Fairlawn, OH) immediately after collection in the field, transported to the lab within eight hours, and immediately oven-dried at 80ºC until weights stabilized. After drying, samples were ground and stored in plastic bottles at -20ºC until the carbohydrate concentrations were analyzed (Kolb and McCormick, 1991).

Carbohydrate analysis

Glucose and starch concentrations were determined for each sample using Sigma® GAGO-20 reagents (Sigma®, St. Louis, MO). Glucose was extracted from tissues using methanol:chloroform:water (MCW, 12:5:3, v/v/v) solution, and 0.5 mL of the supernatant from the extract or glucose standards was mixed with 5 ml of anthrone

reagent (Jaenicke and Thiong'o, 1999). Absorbance of samples and standards were read at 625 nm within 30 minutes using a spectrophotometer (Spectronic 20, Baush & Lomb, Rochester, NY). Glucose concentrations were calculated through standard curve linear regressions and expressed as mg per g of dry weight. Starch content was determined in the remaining pellet using amyloglucosidase, which is the enzyme responsible for the conversion of starch to glucose. The starch standards were prepared using potato starch, and the samples were read at 540 nm within 30 minutes. Starch content was expressed as mg of glucose per g of dry weight. (Haissig and Dickson, 1979; Renaud and Mauffette, 1991).
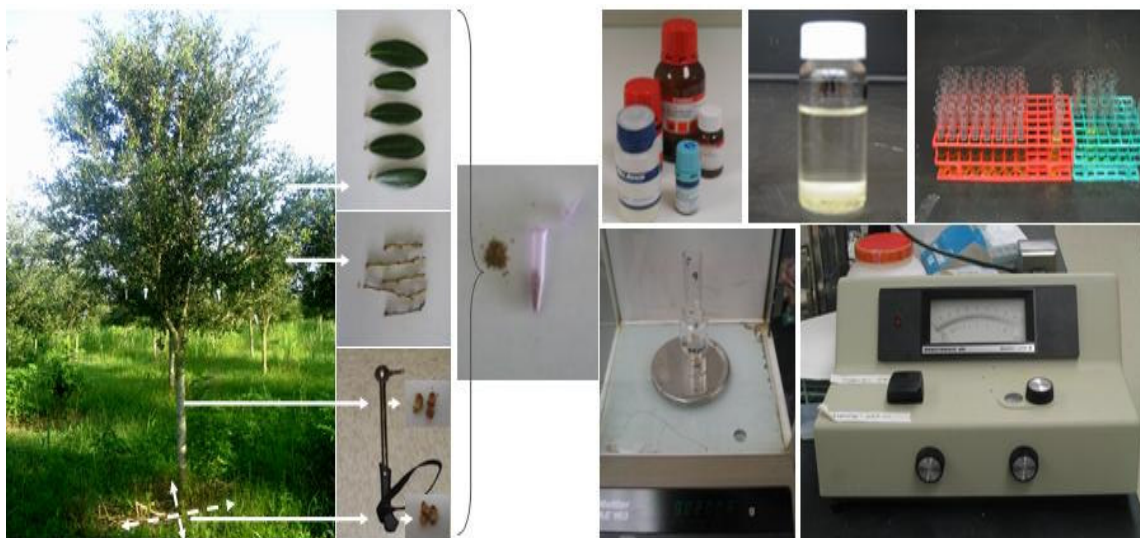
Fig. 2.1. Sample collection and processing of different tissues of live oak.

Data analysis

Data were analyzed by cardinal orientation, type of tissue, and time of year. Results from samples collected from opposite sides of the tree (North-South, and East-West) were pooled in order to compare differences along and between rows in the nursery. Data was analyzed by date considering orientation and type of tissue for comparing results. The data were analyzed with the procedure General Linear Model (GLM) using the Statistical Package for the Social Sciences (SPSS, v. 13) for Windows (SPSS, Chicago, Ill.). Comparisons among tissues and within orientations were performed using Least Significant Differences (LSD) to determine differences in carbohydrate concentration in trees.

Results and Discussion

Annual mean carbohydrate concentrations varied among different tissues in the tree (Table 2.1). Glucose levels in leaves were higher than in twig, root, and trunk tissues which all had approximately half the concentration of glucose present in leaves. For starch, an inverse pattern was found where root and trunk tissues had more than three times the starch content of canopy tissues. This concentration pattern corroborates source-sink mechanisms for carbohydrate partitioning described in other studies (Kaelke and Dawson, 2005; Kaipaiaien and Sofronova, 2003; Taiz and Zeiger, 2006). It is important to point out the role of the trunk as a storage system. Dean (2001) indicated that root allocation can be affected by stem allocation competition due to the fact that stems precede roots on the chain of carbohydrate sinks.

Table 2.1. Annual mean glucose (mg·g^{-1} DW) and starch concentrations (mg·g^{-1} DW of glucose) in different organs from live oak.

Tissue	Glucose^z (mg·g^{-1} DW)	Starch^z (mg·g^{-1} DW of glucose)
Leaves	49.55 a	12.89 b
Twigs	28.80 b	11.41 b
Trunk	22.01 c	38.22 a
Roots	24.02 c	38.98 a

^{z}Means within columns followed by the same letter are not significantly different using LSD at $P \leq 0.001$.

Statistical analysis revealed no differences among tissues collected due to cardinal orientation, even when data from opposing orientations (N-S and E-W) were pooled. These results indicate that future research involving tissue sampling for carbohydrates may not be impacted by cardinal direction when sampling similar tissues at similar heights. However, carbohydrate reserve concentrations varied from the base to the top in the trunks of beech and oak (*Quercus petrea* L. and *Fagus sylvatica* L.) (Babaroux et al., 2003).

Differences in glucose concentrations among tissues varied across seasons (Fig. 2.2). Glucose content was higher in leaves throughout the year than in other tissues tested, except in September when there were no significant differences in glucose levels in leaves and roots. Although leaf glucose levels decreased in September, they were still significantly higher than glucose levels in trunks and twigs. Species such as *Carya illinoinensis* (Wangenh) K. Koch or *Pinus ponderosa* Laws., showed constant values of monosaccharide concentrations during the growing season in different tissues of the tree (Grulke et al., 2001; Kim and Wetzstein, 2005).

Foliar glucose levels decreased in summer and fall, possibly due to the developmental stage of leaves as well as air temperatures. The glucose fraction in roots reached the highest level in the fall which could indicate a higher translocation of sugars to roots for storage purposes (Fig. 2.2). Lower glucose levels in roots were present during the spring-winter period when the roots showed the highest levels of starch. The decrease in glucose content in leaves during fall can be a result of high translocation and replacement of carbohydrates from source to sinks or storing organs (McLaughlin et al., 1980).
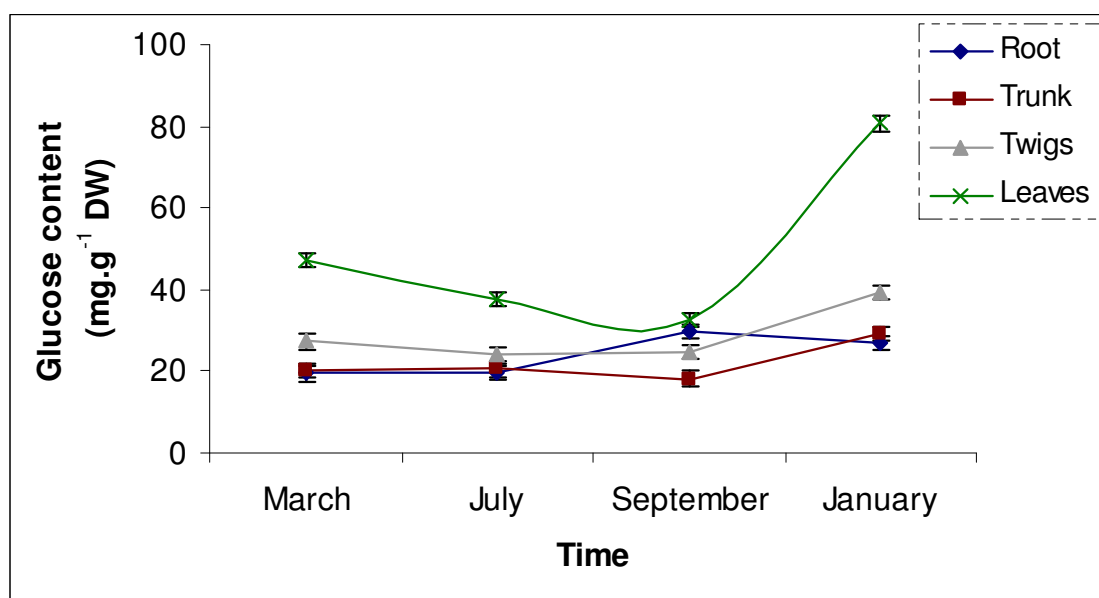
Fig. 2.2. Glucose concentrations (mg·g^{-1} dry weight) within live oak at four different dates. Bars represent the SE of the mean.

Glucose concentrations in leaves might have increased in winter because live oaks maintain live leaves throughout the winter and glucose translocation to storing

tissues decreases. Ludovici et al. (2002) also found a two-to-three-fold increase in winter

glucose concentrations when compared to summer levels in needles of *Pinus taeda* L.

The high levels of glucose in leaves during the winter assessment can also be in response

to environmental factors such as the low temperatures (below 0 ºC) that occurred before

sample collection. Conversion of starch to sugar is a physiological manifestation of cold

hardiness in trees (Levitt, 1980; Nguyen et al., 1990).
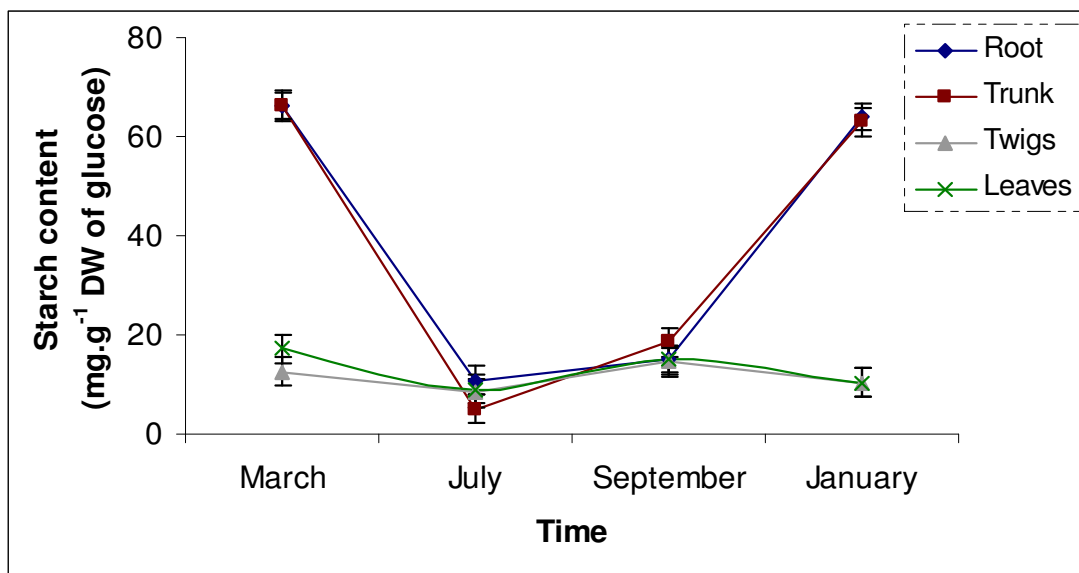
Fig. 2.3. Starch concentrations (mg·g^{-1} DW of glucose) within live oak at four different dates. Bars represent the SE of the mean.

In the case of starch, the highest concentrations were present in roots and trunks

during spring and winter measurements (Fig. 2.3), thus confirming their role in

facilitating carbohydrate storage (Allen et al., 2005). Similar results were found for

trunks and roots of *Q. petrea* which had higher quantities of carbohydrate reserves in

autumn than in late summer (Barbaroux et al., 2003). Trunk tissues showed a depletion

of starch from spring to the summer (Fig. 2.3). Gansert and Sprick (1998) identified starch disintegration during the summer in *F. sylvatica*. Low concentrations of starch were also found during the spring in *P. sylvestris* (Domisch et al*.,* 2002).

Starch concentrations found in root and trunk tissues (Fig. 2.3) emphasize the importance of these two organs as carbohydrate reservoirs during the dormant season. These storage tissues have an important role during the initiation of vegetative growth in the spring. The reduction of starch levels observed in the summer is a consequence of growth and high night temperatures and is associated with the hydrolyzation of stored carbohydrates for growth and maintenance (Kaipiainen and Sofronova, 2003; Pallardy, 2008).

Based on the results of this study, trunk, root, twig, or leaf tissue can be used to monitoring glucose and starch levels throughout the year, considering the seasonal variation in different parts of trees which changes depending on species (Newell et al., 2002). For future studies to monitor carbohydrate levels in trees, additional factors must be considered. For example, twigs exhibited less variation among samples than other tissues that were analyzed (data not shown) and were easy to collect any time of year without causing considerable damage. For leaves, it may be unfeasible to obtain a homogenous sample during the winter or before new growth for deciduous or semi-ever green trees. In the case of collecting roots and trunks, the tree sustains more damage that may disrupt physiological functions. In addition, fine root sampling can be complicated due to fine root loss during the washing process from soil (Kaipiainen and Sofronova, 2003).

CHAPTER III

EFFECTS ON THE MICROBIAL ACTIVITY OF A CLAY SOIL AMENDED WITH

GLUCOSE AND STARCH

Soil microbial activity is highly influenced by carbon substrate in the soil (Jonasson et al., 1996). Plants supply microorganisms with carbon as an energy source through root exudates and root tissues (Ros et al., 2003; Schmidt et al., 1997b). Microorganisms can also be a substrate for other microbes in soils (Wardle and Parkinson, 1990). Most soils usually contain a limited amount of carbon with respect to the requirements by soil microbial populations (Michelsen et al., 1999; Smith and Paul, 1990). Therefore, different types of amendments such as municipal waste and sewage, organic matter, or sugars have been recommended to recover or improve soil quality (Pascual et al., 1999; Pascual et al., 1997; Ros et al., 2003).

Microbial activity can be used as an indicator of soil quality and can be used to assess the effectiveness of soil treatments or cultural practices (Chidthaisong and Conrad, 2000; Ilstedt et al., 2000). Microorganism density affects the rate of organic compound transformation in soils. Microorganism populations play an important role in mineralization, nutrient mobilization, and as a sink of nutrients (Schmidt et al., 2000; Schmidt et al., 1997a). Other benefits of microbial activity are the enhancement of soil structure and the increase in organic matter and symbiotic relationships (Wagner and Wolf, 2005). These beneficial effects of microorganisms have a positive impact on the

growth and vitality of trees and other plants (Harmer and Alexander, 1986; Sanginga et al., 1992).

Soil respiration and microbe plate counting methods have been used to determine microbial activity (Anderson, 1982; Zuberer, 1994). Previous research indicates the relationship between CO_2 emitted by soil respiration and microbial activity (Bååth and Arnebrant, 1994; Ros et al., 2003; Tate, 2000). Plate counting methods only estimate microbial populations able to grow under laboratory conditions, which are a small part (about 1-10%) of the total microbial populations in soils. Despite the limitations, this method is still considered useful for experiments aimed at comparing soil microorganism populations of soils under different treatments (Alexander, 2005; Zuberer, 1994).

Although some research has been conducted to evaluate the effects of glucose added to soil (Schmidt et al., 1997a; Schmidt et al., 1997b), most of the experiments were conducted using low sugar concentrations and on plants grown under laboratory or greenhouse conditions (Ilstedt et al., 2000; Schmidt et al., 2000). Research about the effects of sugar amendments on soil quality can provide information to further research on the effects of carbohydrate applications on tree growth and vitality conducted on larger, field-grown trees. In addition, studies on the use of different carbohydrates may include comparing the effects among simple and complex carbohydrates on microbial activity.

In this study, we evaluated the temporal effects of varying doses and compositions of soil-applied carbohydrates on culturable soil microbial populations and soil respiration under laboratory and field conditions.

Materials and Methods

Treatments and soil sampling

The field portion of the study was conducted on live oaks (*Quercus virginiana*) at a tree nursery located in Monaville, TX (29º57'1.59"N, 96º3'28.73"W). The soil was a deep, moderately well drained, slowly permeable Lake Charles clay. Sugar treatments were applied at the beginning of the study at the concentrations of 40, 80 and 120 g·L^{-1}. The ten treatments were glucose, starch, and a 50:50 glucose and starch mixture (w/w) at the three concentrations and a water control. Treatment concentrations were chosen based on previous research (Jonasson et al*.,* 1996; Percival et al., 2004; Schmidt et al*.,* 2000). The treatments were randomly distributed over 30 trees (three replicates per treatment). The solutions were applied as drenches around the trunk within a 0.5 m radius using 10 L per tree on June 27th, 2005. The volume of solution used was enough to saturate at least the top 15 cm of soil.

Two soil samples (25 mm diameter x 100 mm long) were extracted using a soil probe (AMS Inc. American Fall, ID) within a distance of 0.5 m from the trunk. Coarse and fine roots and macroscopic parts of plants were removed from the soil. Immediately after collection, samples were stored in ice and transported to the laboratory. Once in the laboratory, the samples were stored at 4 ºC until processing (less than 24 hours).

Field studies of microbial populations

Microbial populations were estimated by quantifying recoverable viable microbial populations using the dilution plate count technique. From each soil sample collected after treatment application, 10 g of moist soil was oven-dried at 80 ºC until the

weight stabilized to determine soil moisture content so that microbial populations can be adjusted to a dry-weight basis. Another 10 g subsample was used for preparing the serial dilutions. Each soil sample was diluted in 95 mL blank (water) under a laminar flow hood and shaken vigorously using a vortex mixer (VWR® West Chester, PA) to prepare the first dilution (10^{-1}). Serial dilutions were made from 10^{-2} until 10^{-6} transferring 1 mL aliquot to a 9 mL blank. Different dilutions were used for each microbial population such as 10^{-2} dilution for fungi, 10^{-4} dilution for actinomycetes and 10^{-5} dilution for bacteria. For each dilution, 0.1 mL aliquot was plated and spread on culture media using three replicates (PDA+Rose Bengal; Actinomycete Isolation Agar; Nutrient agar; Difco®) and incubated at 27 ^{o}C for 48 hours (bacteria and fungi) or 5 days (actinomycetes). Colony forming units (CFU) were counted to estimate the total soil microbial populations (Alexander, 2005; Parkinson, 1982; Zuberer, 1994).

Field studies of soil respiration

Microbial activity was estimated by measuring soil respiration using the alkali trap method (Anderson, 1982). From each soil sample, a subsample of 60 g of soil was placed into a glass jar and a small beaker with 3 mL of 1.0N NaOH was placed over the soil in the jar. Jar lids were tightened to avoid gas leakage and incubated at 27 °C for 24 hours. After incubation, two drops of phenolphthalein and 1 mL $BaCl_2$ (50% solution) were added to precipitate the carbonates. Samples were then titrated with 1.0N M HCl, and CO_2 evolution was estimated based on the amount of HCl required in the titration (titration was obtained once the pink color of the solution disappeared). The amount of CO_2 released by blank samples was estimated by repeating the same process in jars

without soil. The amount of CO_2 evolved from soil was then determined following the calculations according to Anderson (1982).

Soil samples for measuring soil respiration, as CO_2 evolution, and total microbial populations were collected weekly for a period of six weeks. The average air temperature in the field during the study period was 23.3 ℃, with a maximum of 34.8 ℃ and a minimum of 29.0 ℃. An additional study for soil respiration determination was conducted at another site in the nursery with similar soil conditions during the winter of 2006 (application on January 30) and compared to the results from summer 2005. In the case of the winter period, the average temperature was 17.6 ℃, with a maximum of 23.4 ℃ and a minimum of 11.8 ℃.

Laboratory studies of microbial populations and soil respiration

To study soil respiration and microbial populations under more controlled temperatures, another experiment was performed using soil samples collected from the nursery and evaluated under laboratory conditions. As in the prior two experiments, samples were collected from the top 15 cm of soil at the nursery and placed in small plastic containers 18Lx12Wx7D cm (Rubbermaid®; Wooster, OH). The soil samples were transported to the laboratory and kept in an incubator at 27 ℃ under dark conditions throughout the essay. The same carbohydrate concentrations at proportional solution amounts (200 mL) as those used in the field experiment were applied at the beginning of the experiment. The soil moisture was kept constant throughout the experiment by weighing the container every 48 hrs and adding distilled water as needed.

Soil respiration and total microbial populations were measured weekly for nine weeks using the same methodology previously described.

Data analysis

The treatments were distributed under a complete randomized design with three replicates including time in the analysis. The data were analyzed using type III sums of squares in the GLM procedure using SPSS v13. When significant treatment effects were detected, the treatment means were tested using Dunnett's one tailed t-test for differences from the control at a significance level of 0.05.

Results and Discussion

Field studies of microbial populations

Recoverable viable microbial populations from the field studies had an overall mean during the experiment of $9.8x10^3$ CFU·g^{-1} soil ($\pm 4x10^3$ standard deviation, SD) for fungi, $8.5x10^6$ CFU·g^{-1} soil ($\pm 3.7x10^6$ SD) for bacteria, and $10.7x10^4$ CFU·g^{-1} soil ($\pm 5.3x10^4$ SD) for actinomycetes. There was high variability among replicates when enumerating microbial populations using the plate counting technique, which affected the ability to identify differences among treatments in most cases. The application of 120 g·L^{-1} of glucose exhibited higher numbers of fungi in the fourth and fifth weeks, and the 120 g·L^{-1} of the 50:50 mixture of glucose and starch showed considerably higher numbers of CFUs for fungi in the fifth week (Table 3.1).

Table 3.1. Average Colony Forming Units (CFU·g^{-1} soil) per gram of soil (± standard deviation) of fungi, bacteria, and actinomycetes in soil after being amended with solutions of glucose, starch, and a 50:50 mixture at different concentrations (0 40, 80, and 120 g·L^{-1}) under field conditions.

Treatment	Fungi (10^3 CFU·g^{-1} soil)						
	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Control	2.8±0.3	4.5±1.6	10.1±0.8	11.3±1.4	8.9±3.5	8.5±0.6	11.0±3.7
Glucose 40	1.8±0.1	8.0±2.7	9.3±1.8	13.5±1.4	9.1±0.4	9.7±1.4	12.4±1.6
Glucose 80	4.5±1.1	8.5±2.2	15.9±4.8	13.2±2.0	9.1±2.6	12.6±2.9	12.1±4.9
Glucose 120	6.7±3.0	11.4±1.8	17.3±0.5	14.1±2.3	17.3±3.1	17.0±5.5	18.6±3.0
Starch 40	3.6±0.6	6.1±1.0	12.2±4.6	11.1±0.6	6.5±1.5	9.5±0.5	6.9±2.2
Starch 80	2.4±0.6	6.7±1.8	12.4±1.4	11.3±3.3	4.3±1.6	6.8±1.4	9.2±2.2
Starch 120	3.6±1.5	9.2±3.4	15.5±1.3	10.9±0.6	11.8±2.0	10.4±1.6	9.0±1.4
Mixture 40	3.9±0.2	10.6±1.3	9.2±1.3	16.7±2.9	7.0±2.1	8.7±1.9	10.2±0.5
Mixture 80	2.8±.6	9.9±2.1	13.8±2.0	13.4±0.8	6.3±1.9	12.3±3.2	8.9±3.8
Mixture 120	5.1±1.5	7.5±2.9	9.4±2.5	12.0±2.3	9.0±1.5	18.3±3.7	14.7±4.2

	Bacteria (10^6 CFU·g^{-1} soil)						
	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Control	8.3±1.4	3.6±1.2	3.3±1.3	10.2±2.6	8.3±2.1	10.2±1.4	15.6±5.4
Glucose 40	3.6±1.9	7.3±1.6	4.2±1.1	7.7±0.8	6.7±0.8	8.0±1.7	11.6±4.1
Glucose 80	9.8±4.2	4.2±1.1	5.2±2.0	3.3±0.8	6.6±1.8	10.9±2.1	14.9±4.4
Glucose 120	9.0±1.5	7.4±2.3	10.9±1.3	6.4±0.7	12.3±3.5	9.2±4.1	13.1±2.5
Starch 40	5.2±2.3	7.1±2.3	3.8±3.5	3.5±0.2	5.0±1.6	8.0±2.8	11.5±3.7
Starch 80	15.1±1.3	5.5±1.4	6.2±1.6	4.9±0.7	11.7±0.4	12.2±1.9	15.1±3.2
Starch 120	4.6±2.6	4.7±1.2	7.3±2.2	5.7±2.1	9.9±1.8	9.6±0.6	16.3±3.2
Mixture 40	7.1±1.1	6.4±1.3	5.5±1.4	5.2±0.8	8.5±2.0	10.4±4.2	13.4±4.2
Mixture 80	2.4±0.3	6.3±4.5	5.8±1.3	4.6±1.8	10.2±4.7	14.6±1.4	12.9±2.2
Mixture 120	9.2±1.4	7.1±4.0	13.8±0.2	8.5±2.0	7.6±0.5	16.9±1.3	15.6±2.2

	Actinomycete (10^4 CFU·g^{-1} soil)						
	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Control	9.2±4.0	7.1±5.1	14.3±2.3	11.3±0.4	5.6±3.8	7.5±1.5	5.2±1.8
Glucose 40	5.7±2.0	7.9±4.4	21.7±5.5	12.9±4.1	6.7±3.6	6.9±2.4	10.7±6.8
Glucose 80	9.9±2.1	6.8±2.0	16.4±5.8	5.4±2.2	5.7±1.3	8.3±3.0	5.9±2.4
Glucose 120	11.0±2.5	8.5±1.6	17.8±7.3	7.6±6.3	13.7±3.3	7.0±2.3	10.2±1.9
Starch 40	5.3±0.9	8.6±1.5	17.5±4.5	10.1±2.1	7.6±1.8	8.0±1.3	3.3±1.1
Starch 80	9.9±1.8	14.4±4.4	13.7±7.3	12.3±2.6	7.0±2.0	8.5±1.5	10.4±7.6
Starch 120	8.1±4.8	13.1±5.8	22.7±4.3	12.5±4.1	12.2±1.6	9.5±0.6	8.7±4.4
Mixture 40	10.4±3.1	10.0±5.7	16.8±2.9	17.4±2.2	10.7±5.3	7.4±2.5	10.5±2.5
Mixture 80	5.4±2.1	16.3±4.6	27.8±4.0	9.9±1.7	6.2±3.4	12.2±3.5	5.1±3.5
Mixture 120	10.3±2.4	8.0±2.5	34.1±4.8	12.6±2.9	8.1±4.1	10.8±4.4	6.0±0.7

Recoverable bacteria and actinomycetes had higher CFUs in the samples collected the second week in soil treated with 120 g·L^{-1} of a 50:50 mixture (glucose and starch) (Table 3.1). Although all treatments demonstrated a visible increase in CFUs, only the highest concentrations affected in some degree soil microbial populations on different dates. The lack of significant differences ($P \geq 0.10$) found in treatments with carbohydrate concentrations was due to the high degree of variation among data.

Laboratory studies of microbial populations

For experiments conducted in the laboratory, the overall mean values of the recoverable viable microbial populations were 13×10^3 CFU·g^{-1} soil ($\pm 3.1 \times 10^3$ SD) for fungi, 15×10^6 CFU·g^{-1} soil ($\pm 4.8 \times 10^6$ SD) for bacteria, and 8×10^4 CFU·g^{-1} soil ($\pm 3.5 \times 10^4$ SD) for actinomycetes. As with the results from the field experiments, the high variation presented by the data did not allow the detection of statistically significant differences or identify a particular trend for the microbial populations (Table 3.2). The lack of correlation between field and laboratory results could have been the result of the extraction and storage of soil samples in the lab for the laboratory experiment as opposed to the field experiment where samples remained intact in the field until they were evaluated. Fluctuations in temperatures and moisture experienced under field conditions may have also influenced the differences observed.

Table 3.2. Average Colony Forming Units (CFU·g^{-1} soil) per gram of soil (± standard deviation) of fungi, bacteria, and actinomycetes in soil after being amended with solutions of glucose, starch, and a 50:50 mixture at different concentrations (0, 40, 80, and 120 g·L^{-1}) under laboratory conditions.

Treatment	Fungi (10^3 CFU·g^{-1} soil)						
	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Control	10.0±1.2	12.9±5.4	12.7±5.9	14.4±1.2	11.9±2.5	13.1±3.5	14.4±5.0
Glucose 40	13.0±1.3	12.3±2.9	13.2±7.4	12.9±3.5	11.8±6.2	12.3±3.1	12.9±1.9
Glucose 80	12.9±1.6	11.8±1.8	7.2±0.6	15.5±2.0	18.0±3.0	17.9±4.6	17.9±6.7
Glucose 120	9.6±2.7	6.5±1.8	9.1±4.4	11.0±3.0	14.8±5.4	12.6±4.6	10.5±3.6
Starch 40	13.4±1.2	12.6±3.0	12.4±5.6	16.5±6.3	15.1±2.5	15.8±3.1	16.5±4.1
Starch 80	11.2±1.9	11.7±4.0	7.5±2.0	13.9±2.3	14.0±0.7	14.1±5.1	14.2±8.3
Starch 120	9.4±0.4	9.7±2.7	11.8±3.5	10.2±3.4	13.0±3.2	11.5±3.1	10.0±3.0
Mixture 40	12.7±1.1	10.0±1.9	10.1±2.7	14.0±6.5	15.6±1.1	15.1±2.1	14.6±2.6
Mixture 80	14.8±4.4	16.7±6.1	15.0±2.6	15.4±4.6	13.0±2.6	15.7±4.6	18.4±6.4
Mixture 120	13.5±1.3	6.7±1.8	12.6±3.2	15.0±2.6	14.8±1.1	10.8±1.4	6.9±1.7

	Bacteria (10^6 CFU·g^{-1} soil)						
	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Control	10.8±2.8	14.9±1.1	22.3±0.5	19.6±2.1	18.8±9.0	13.7±5.1	8.7±4.4
Glucose 40	8.4±3.6	14.6±3.5	17.5±4.2	19.1±2.0	14.0±5.3	15.5±6.2	17.1±9.9
Glucose 80	8.9±2.9	21.0±2.5	15.0±3.5	12.2±9.3	13.3±3.0	13.1±2.1	13.0±2.3
Glucose 120	6.7±4.3	19.8±3.9	23.3±0.2	26.2±2.0	18.8±0.7	14.4±0.5	10.0±0.3
Starch 40	6.6±1.3	18.0±0.8	17.0±4.1	19.3±4.1	15.9±0.9	14.1±1.3	12.3±3.4
Starch 80	9.4±0.8	15.2±2.0	18.4±1.1	17.5±3.3	10.1±2.1	11.3±1.8	12.5±1.5
Starch 120	9.7±3.5	16.5±7.6	22.4±6.5	13.3±7.2	10.3±2.3	11.0±4.1	11.9±5.2
Mixture 40	9.1±1.8	16.2±3.8	23.5±2.5	16.8±2.5	13.8±0.9	15.6±2.5	17.2±4.1
Mixture 80	9.5±1.0	15.2±4.1	19.8±3.9	20.1±1.1	15.5±0.7	14.0±1.1	12.6±1.4
Mixture 120	10.5±1.4	17.0±2.5	20.1±1.3	24.8±5.1	22.6±2.4	16.4±2.9	10.0±3.1

	Actinomycete (10^4 CFU·g^{-1} soil)						
	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Control	4.3±1.5	9.9±5.7	8.9±1.5	8.5±1.5	5.5±0.6	8.4±2.1	11.5±5.6
Glucose 40	6.0±3.0	10.1±1.8	7.5±4.4	4.7±1.6	5.1±3.8	9.2±2.8	13.1±2.5
Glucose 80	6.2±3.0	5.1±1.8	2.5±1.8	7.2±3.1	6.4±2.0	10.0±3.9	13.6±5.7
Glucose 120	8.0±3.9	4.1±1.5	7.2±5.7	3.8±1.2	2.7±1.2	4.5±1.9	5.3±2.4
Starch 40	10.0±3.9	5.0±2.7	7.8±4.5	8.0±2.2	4.6±2.1	8.7±3.2	12.9±4.1
Starch 80	7.1±0.7	6.4±1.7	5.8±2.1	5.9±2.1	5.6±2.2	9.2±3.8	12.7±5.1
Starch 120	8.7±1.9	8.0±6.7	9.1±5.0	3.8±2.0	5.8±0.8	8.0±2.1	10.6±4.9
Mixture 40	8.2±4.0	8.5±3.3	7.5±2.1	6.0±0.7	6.5±2.8	11.1±3.9	16.4±5.6
Mixture 80	10.9±3.5	7.7±4.0	11.7±8.9	5.9±1.5	4.2±1.1	10.6±4.1	16.8±6.7
Mixture 120	6.4±4.0	5.9±0.7	10.5±4.7	6.7±4.9	3.3±2.5	10.5±1.9	18.4±1.1

Field studies of soil respiration

Soil respiration rates were differed after being treated with the different carbohydrate amendments. Respiration rates significantly ($P<0.05$) increased one week

after applying glucose (Fig. 3.1A). Previous research showed that measurement of CO_2 evolution is a method generally convenient, rapid, and accurate in the assessment of soil microbial activity (Anderson, 1982; Bååth and Arnebrant, 1994; Tate, 2000). Higher rates of respiration recorded soon after glucose application indicates how the substrate was easily utilized by microbes (Schmidt et al., 1997a). The increase in respiration rates for the 120 g·L^{-1} glucose concentration remained high until the third week after treatments under field conditions, while the effect by the lower glucose concentrations (40 and 80 g·L^{-1}) lasted only until the second week. Respiration with glucose treatments showed trends similar to those found in previous research using soil amended with glucose (Jonasson et al., 1996; Wu et al., 1993). Dassonville et al. (2004) found that the effect of glucose applications (20 g·L^{-1}) disappeared in about a week under anaerobic conditions.

When starch was applied at 40 and 80 g·L^{-1}, there was a significant increase ($P<0.05$) in respiration during the second week after treatments (Fig. 3.1B). The increase for the highest concentration (120 g·L^{-1}) of starch was delayed until the fourth and fifth weeks. These results may have been due to the higher amounts of starch, which altered the carbon/nitrogen ratio in the soil or by the low amount of starch-degrading enzymes present in the soil (Wagner and Wolf, 2005). Starch applications had a less pronounced effect than glucose during the first few weeks after application, but the effect was prolonged in the highest concentrations. Previous research found than 10 to 20% of the starch was still present in soil 6 weeks after application (Papavizas et al., 1968).

Fig. 3.1. Respiration in soil samples amended with glucose (A), starch (B), and a 50:50 mixture (C) at different concentrations (0, 40, 80, and 120 g·L^{-1}) under field conditions (summer 2005). *Indicates significant differences against the control on each date using Dunnet's test ($P<0.05$).

Respiration rate in soil treated with the 50:50 carbohydrate mixture was significantly higher than the control ($P<0.05$) after two weeks for the 80 and 120g·L^{-1} concentrations. This effect lasted until the fifth week only for the 120 g·L^{-1} carbohydrate mixture (Fig. 3.1C). The increase in respiration rate could have been affected mainly by the glucose portion of these treatments since the rates are between those observed for the glucose and starch only treatments. The lowest mixture concentration (40 g·L^{-1}) did not have a significant impact on respiration rate. As it was mentioned before, the effect of the glucose amount used in low concentrations disappeared in about a week after the treatment (Cheshire, 1979; Dassonville et al., 2004), while the small amount of starch (20 g·L^{-1}) in the lowest mixture concentration did not have a significant effect.

Laboratory studies of soil respiration

Respiration values for samples that were kept under laboratory conditions were also differently ($P<0.05$) affected by the carbohydrate amendments through time (Fig. 3.2A). The respiration values after a week of the glucose applications showed higher values than the field experiment. The reasons for this could be due to differences in temperature (Tate, 2000) or the time of year that samples were collected from the field. In addition, the plastic containers used in the laboratory experiment did not permit the loss of sugars by leaching as could have happened in the field. The higher concentrations showed higher respiration rates until the fourth week in the case of glucose at 80 g·L^{-1} and the fifth week for the 120 g·L^{-1} concentration. The fact that the significant effect was prolonged under laboratory conditions can also be caused by the reduction in microbial

colonies as a result of the change in growth conditions as has been reported in other laboratory methods (Zuberer, 1994).

The starch treatments did not show a significant effect on respiration during the first weeks (Fig. 3.2B); however, the starch at 120 g·L^{-1} showed a significant increase in respiration values during the sixth week, and the effect lasted until the end of the experiment (week 9). Previous research revealed a lag phase after the addition of organic polymers due to the synthesis and activity of extracellular enzymes that participated in the degradation of more complex compounds (Ros et al., 2003). Guggenberger et al. (1999) pointed out that the slow decomposition rate of starch was due to low nutrient availability, mainly N-limited. Starch results indicated the response of soil microbial populations to different substrates that are not as easily metabolized as glucose; therefore, the process might imply the participation of different microorganisms in the substrate metabolism. Regardless of the type of substrate added, the values of respiration decreased with time as a consequence of the substrate metabolism by microorganisms (Dassonville et al., 2004).

The mixtures treatments also increased respiration. The effect lasted until the second week for the 80 g·L^{-1} concentration and until the fourth week for the 120 g·L^{-1} concentration. The mixtures at 120 and 80 g·L^{-1} also exhibited an increase in respiration during the eighth and ninth week (Fig. 3.2C). This effect could be due to starch utilization as seen in the starch treatment. When compared to the field experiment, carbohydrate metabolization may be temperature-dependent with the simpler sugars

(glucose) being broken down quicker and at lower temperatures than the more complex

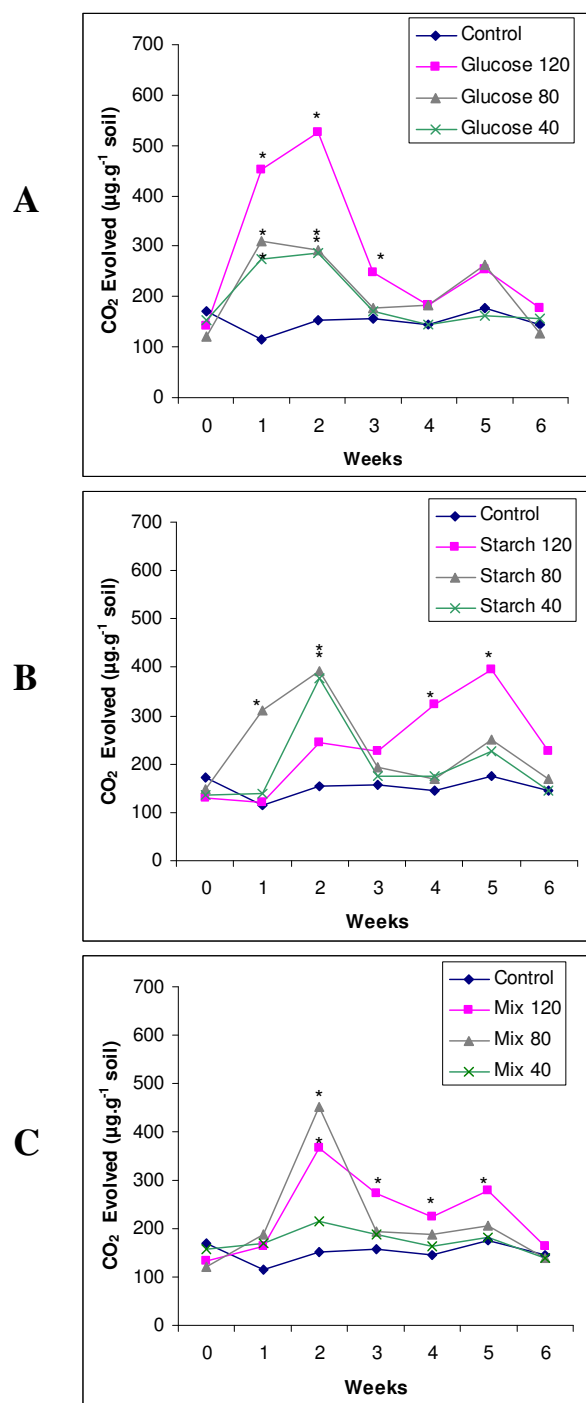carbohydrates (starch), which likely required higher temperatures, more time, or both.

Fig. 3.2. Respiration in soil samples amended with glucose (A), starch (B), and a 50:50 mixture (C) at different concentrations (0, 40, 80, and 120 $g \cdot L^{-1}$) under laboratory conditions (summer 2005). * indicates significant differences against the control using Dunnet's test ($P<0.05$).

To determine the impact of temperature and season on respiration rates, additional treated samples were collected at the field in winter 2006. Respiration values after glucose and starch amendments during the winter 2006 showed trends similar to the summer 2005 results. The highest concentration of glucose (120 g·L^{-1}) increased soil respiration during the first and third week (Fig. 3.3A). Due to the high variability of results in the first measurements, significant differences were not detected in the lowest concentration (40 g·L^{-1}).

The 120 g·L^{-1} starch concentration showed significant differences in the fifth through the ninth week. The high variability and decrease in the effect of the starch and glucose as soil amendments could be affected by the rainfall presented during the first weeks after treatments application; however, a significant effect of starch on microbial activity was still showed in the ninth week. Previous research indicated that microbial activity can be stimulated for weeks by incorporation of carbon sources (Ros et al., 2003).

In the case of the 50:50 mixtures (glucose:starch), the treatments showed significant differences during the first and third weeks after sugar applications for the 80 and 120 g·L^{-1} concentrations while the lowest concentration (40g·L^{-1}) did not show significant differences. The lowest amounts of glucose at a rate of 50% were easily used by microorganisms or lost for environmental factors or irrigation while the starch amount at 50% did not cause any significant effect on soil respiration.
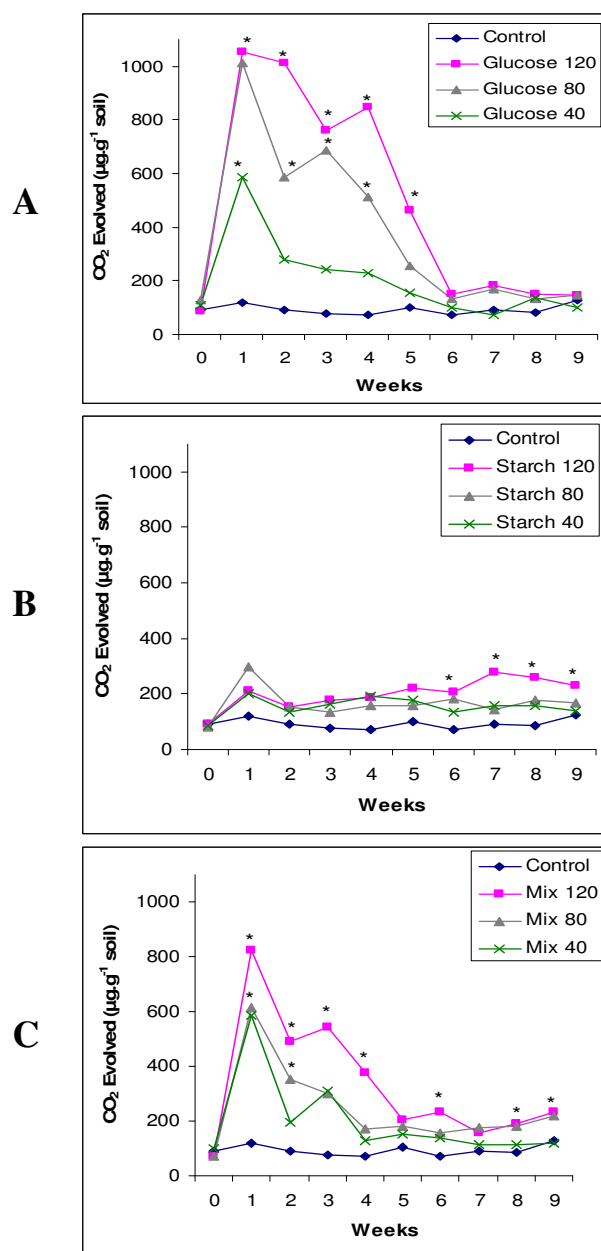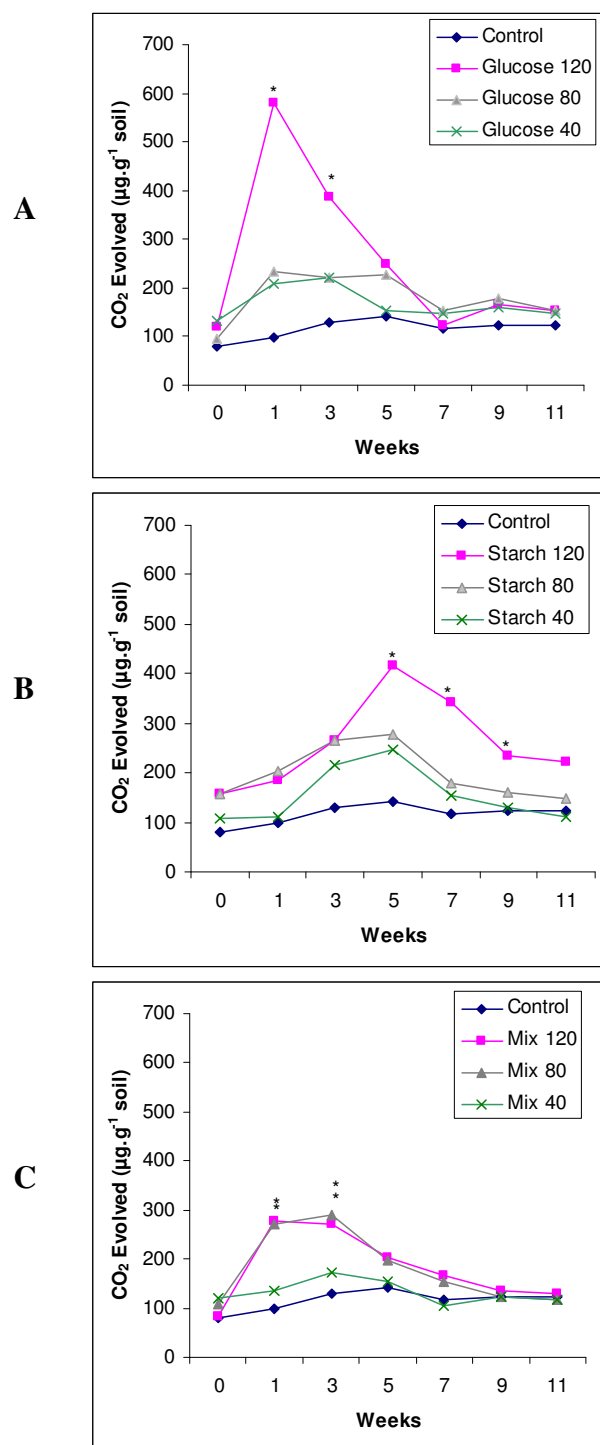
Fig. 3.3. Respiration in soil samples amended with glucose (A), starch (B), and a 50:50 mixture (C) at different concentrations (0, 40, 80, and 120 g·L^{-1}) under field conditions during the winter 2006. * indicates significant differences against the control using Dunnet's test ($P<0.05$).

In both of the methods used in this paper, understanding the impact of exogenously applied carbohydrates proved difficult. Although respiration increased dramatically thus indicating an increase in microbial activity, there was not a significant increase in soil microbial CFUs. The lack of correlation between number of microorganisms and microbial activity could have been due to the intrinsic characteristics of the plate counting technique. The technique usually estimates a small part of the total population and can fail to extract all cells from soil particles (Zuberer, 1994). It is also possible that certain microorganisms may have been preferentially selected due to their size, configuration, or vast numbers during the dilution process, which might have resulted in the evaluation of microbes that were not affected by sugars. In addition, microorganisms may have been affected by media, incubation temperature, and other environmental constraints (Alexander, 2005).

Another reason for the alteration of microbial activity when soil amendments have been used may be due to a change in the soil's chemical properties. Jonasson et al. (1996) found that the addition of sugars to the soil did not alter the soil pH. Wu et al. (1993) found that a high concentration of glucose can have a toxic effect on native microbial biomass. Consequently, the observed increase in microbial activity was caused by the use of dead microbial biomass on behalf of surviving microorganisms. However, the results from this study did not indicate a reduction of culturable soil microorganisms under laboratory conditions due to carbohydrate applications. The different soil respiration responses to the amendments may be explained by varying substrate utilization by bacteria and fungi in the soil. Hu and van Bruggen (1997) found a

multiphase curve response by soil microbes when cellulose was added to the soil. In general, the effect on soil amended with carbohydrates lasts until the carbon source is metabolized, and then the soil seems to return to the initial conditions. Tate (2000) indicated that in many cases the elimination of the treatment effect or the effect of environmental factors can cause the microbial activity to return to the initial levels.

There is still much that needs to be understood about the potential for using carbohydrates to improve the health of soils and plants. The modification of microbial activity through the use of sugar should be done carefully because carbohydrate introduction may negatively impact soils when pathogenic microorganisms are present. The addition of carbonaceous materials, such as starch, to the soil has been investigated against fusariosis (Gupta, 1986; Tramier and Antonini, 1977).

The increase in respiration as a product of exogenous carbohydrates may indicate how carbon sources are a limiting factor for microorganisms in soil (Schmidt et al., 2000). In our study, exogenously-applied carbohydrates had differing effects on microbial activity as measured through soil respiration. In general, soil amended with glucose showed a rapid and short-lived increase in respiration. The starch effect lasted longer but with a lower effect, and the 50:50 mixtures (glucose:starch) showed an intermediate effect as a result of the influence of both types of carbohydrates. The results corroborated that glucose and starch as soil amendments can be used as an easy and practical way to improve microbial activity in soils. However, the effects last until carbohydrates are metabolized by soil microorganisms.

CHAPTER IV

INVESTIGATIONS OF EXOGENOUS APPLICATIONS OF CARBOHYDRATES

ON GROWTH AND VITALITY OF LIVE OAKS

Urban trees frequently grow under stressful conditions (soil compaction, mechanical damages, drought, high temperatures, pollution, etc.) and arborists make use of several practices to maintain or improve tree vitality and growth. Several products, such as growth regulators, fertilizers, root inoculants, and recently carbohydrates, have been recommended for improving tree health (Harris et al., 2004; Percival et al., 2004). Carbohydrates are the main source of energy for plants and are primarily produced by photosynthesis and broken down by respiration to produce energy (Taiz and Zeiger, 2006).

Carbohydrates applied as soil drenches can improve soil conditions or be taken up by roots, incorporated into the tree system, and become an alternative source of energy (Cheshire, 1979; Stubbs et al., 2004). Recently, sugar applications have been recommended as a potential arboricultural practice (Percival and Fraser, 2005) to improve plant performance (Jonasson et al., 1996) or as a way to supplement the photosynthetic energy for plants (Percival and Smiley, 2002).

Growth is a measurable tree characteristic that has been used in experiments to assess the responses from treatments and can be used as a way to evaluate vitality (Polak et al., 2006). Tree height, trunk diameter, and root growth are some growth variables that have been used as an indicator of tree vitality (Dobbertin, 2005). Measuring tree height for species with a decurrent crown pattern, no-main trunk, is challenging because of the

presence on one or more codominant leaders. The use of digital photography to measure overall canopy growth over time can be an option for these species (Montes et al*.,* 2000; Taylor, 1998). The evaluation of total root growth is a relatively easy practice for small seedlings in the nursery, but this technique increases in difficulty as plant size increases. For large trees, samples of the root system have been used as a way to compare root growth of trees under varying environments (Cheng and Bledsoe, 2002; Madji et al*.,* 2005). Although these root measurements provide only partial fine root growth estimates, researchers have still been able to use the data for comparing the effects of sugar treatments (Percival et al*.,* 2004).

Carbohydrates are translocated and stored in different tissues ruled by sink-source relationships (Taiz and Zeiger, 2006). The analysis of carbohydrate content in tissues has been used to assess the impact of environmental factors or chemical applications to trees (Domisch et al., 2002; Gansert and Sprick, 1998; Samuelson and Kelly, 1996). The carbohydrate content in different plant tissues is generally determined colorimetrically in a laboratory. The simple sugars are extracted from tissue in methanol:chloroform:water (MCW) solvents by centrifugation, while insoluble sugars are determined in the remaining pellet using the enzyme amyloglucosidase. (Haising and Dickson, 1979; Renaud and Mauffette, 1991). Unfortunately, there is little research available concerning the effects of exogenous carbohydrate applications on trees in urban environments.

Chlorophyll fluorescence is another variable associated with the process of photosynthesis (Maxwell and Johnson, 2000). The fluorescence response is induced by a

red light of 600 W/m^2 intensity (Percival, 2004; Percival and Fraser, 2001). Chlorophyll fluorescence parameters have been used to detect physiological stress on trees (Sestak and Stiffel, 1997), and the ratio between variable and maximal fluorescence (Fv/Fm) has been used as indicator of tree vitality (Percival and Sheriffs, 2002). Carbon assimilation rate and chlorophyll fluorescence might provide more evidence about the feasibility of using carbohydrates application in the soil around urban trees.

Soil microorganisms are another important component in the use of carbohydrate applications in the soil and the impact on the total tree system. The effect of carbohydrates on microbial activity depends on the type of carbohydrate, leaching through the soil profile, potential root uptake, or microbe's degradation or sequestration (Jonasson et al., 1996; Schmidt et al., 1997b; Wagner and Wolf, 2005). Applications of carbohydrates to the soil as drenches can increase microbial activity in the soil (Illeris and Jonasson, 1999). Microbial activity can affect the rate of nutrient uptake by tree root systems making elements available to plants (Michelsen et al., 1999). However, the increase of microbial populations can also immobilize nutrients in the soil and affect growth rates of plants (Jonasson et al., 1996). Evaluating microbial activity after carbohydrate applications as well as nutrient analysis of soil and leaves will help determine and interpret the effect of continuous carbohydrate applications to the soil and tree nutritional levels.

Uptake of carbohydrates by roots can be affected by different factors, such as microbial populations, infiltration, and root exudates (Jonasson et al., 1996; Wagner and Wolf, 2005). Some researches have used radiocarbon (^{14}C) in order to trace exogenous

applications of carbon compounds and evaluate the allocation or uptake rate (Riek et al.*,* 1997; Srivastava and Srivastava, 2006; Tarpley et al., 1994). These studies have to be developed in laboratories under controlled conditions, which make them unsuitable for use under field conditions. Carbon isotope composition ($\delta^{13}C$) of plant tissue or soil organic matter has been used in ecological studies to determine the C_3 and C_4 plant composition of ecosystems (Fotelli et al.*,* 2003; Stock et al.*,* 2004). Values of $\delta^{13}C$ for C_3 plants range from -20 to -34‰ and for C_4 plants from -9 to 16.8‰ (Pate and Dawson, 1999). Live oaks are C_3 plants, whereas corn and sugarcane are C_4 plants. Considering that carbon isotope ratio ($\delta^{13}C$) has been used to determine type of plant between C_3 and C_4 plants (Stock et al.*,* 2004), applying sugar from cane or corn to a C_3 plant, like live oak, may make it possible to determine uptake of exogenous sugar applications by assessing $\delta^{13}C$ values from tissues of C_3 plants treated with sugars from C_4 plants. The main objectives of this research were to evaluate the effects of exogenous applications of carbohydrates on growth and vitality of live oaks as well as determine the lasting effect of carbohydrate applications on trees and soil.

Materials and Methods

Sixty, field-grown live oaks (*Quercus virginiana*), approximately 10-cm trunk caliper measured 30 cm above ground, were randomly selected from within a nursery at Monaville, TX. (29º57'1.59"N, 96º3'28.73"W). Trees selected in 2004 were planted in 1999 at 5-m spacing and grown under similar conditions.

Solutions of glucose, starch, or a 50:50 mixture of each derived from corn were applied to the soil as a drench around the trunk within a 0.5 m radius from the trunk at 0,

40, 80, and 120 $g \cdot L^{-1}$. The carbohydrate concentrations were chosen according to previous research concerning carbohydrate applications on trees (Percival and Fraser, 2005; Percival et al., 2004; Percival and Smiley, 2002). Experimental trees were separated by at least one untreated tree to serve as a buffer between treatments. Each tree received 10 L of solution per application with the purpose of saturating at least the top 15 cm of soil where the majority of the fine roots are located (Harris et al., 2004). The applications were done during the summer of 2004, winter 2004, spring 2005, summer 2005 and winter 2005.

Trunk diameters were recorded each season throughout the experiment at 30 cm above the ground using a diameter tape measure (Forestry Suppliers Inc.; Jackson, MS). Photographs were taken with a digital camera (Cannon Powershot A75; Lake Success, NY) of all trees against a white background from two-different directions at the same distance and angle. The TIFF-formatted images (2048x1536 pixels) were analyzed using the digital imaging software ImageJ (Abramoff et al., 2004) to quantify canopy density over time and evaluate green intensity. To avoid the possible effect of initial differences among trees, the growth index was calculated per year by dividing the absolute increment in a year by the first measurement (Arnold et al., 2007). The growth index values were used for statistical analysis.

In order to evaluate root growth, four holes (15 cm deep x 6 cm diameter) were drilled on a square pattern at 0.5 m from the trunk of all trees. These holes were refilled with a sandy soil before the first treatment application to measure new root growth into the soil cores and to make it easier to extract roots from the samples (Percival et al.,

2004). Herbicide (glyphosate) was applied periodically during the experiment to eliminate weeds. Soil samples were extracted one year after treatment applications using a 15 cm deep x 6 cm diameter core sampler (AMS Inc., American Fall, ID). The soil cores were washed to extract the roots, and roots were kept in a cold room at 4 ℃ until measurements were taken. Root dry weight was measured with a precision scale (Mettler AE163; Columbus, OH) after area, length and diameter of root samples were measured using the WinRhizo© software (Regent Instruments Inc., Québec, Canada). The holes were refilled with sandy soil after the first sample and root samples were collected in the same location during the second year.

Twig samples were analyzed for carbohydrate content every four months. The use of twig tissue was based on results from preliminary experiments (data not shown). Samples were collected from the lower two-thirds of the canopy on all trees. Glucose and starch contents were determined for each sample using Sigma® GAGO-20 reagents (Sigma®, St. Louis, MO). Glucose was extracted from tissue with methanol:chloroform:water (MCW, 12:5:3, v/v/v) solution after centrifugation at 2800 rpm. A 0.5-mL aliquot of the extract and the glucose standards were mixed with 5 mL of anthrone reagent (Jaenicke and Thiong'o, 1999). Starch content was determined in the remaining pellet using the enzyme amyloglucosidase. Absorbance of samples and standards was read within 30 minutes with a spectrophotometer (Spectronic 20, Baush & Lomb, Rochester, NY) set at 625 nm for glucose and 540 nm for starch (Haissig and Dickson, 1979; Renaud and Mauffette, 1991). After 18 months, woody root samples were also collected and carbohydrate content was analyzed.

Net carbon assimilation was measured in each treatment using a portable photosynthesis system (LI-6200, Li-Cor®, Lincoln, NE). Net carbon assimilation was measured during the morning of sunny days on leaves on the southern side of the canopy. Three leaves per tree were measured from the lowest third of the canopy. To avoid differences between measurements due to time, only three trees per treatment were measured.

Chlorophyll fluorescence was measured using a HandyPEA® portable fluorescence spectrometer (Hansatech Instruments Ltd, King's Lynn, UK) every four months. Ten leaves from the lower two-thirds of the canopy were adapted to darkness by attaching exclusion clips to the leaf surface. Preliminary tests indicated that the time necessary to achieve leaf dark adaptation was 25 minutes. After the darkness period, measurements were taken using the HandyPEA® (Percival and Fraser, 2005; Percival and Fraser, 2001).

Potential carbohydrate uptake by trees under field conditions was determined by carbon isotope ratio ($\delta^{13}C$). Tissue samples from twigs and roots were collected from water control, glucose (40 and 120g·L^{-1}) and starch (40 and 120g·L^{-1}) treatments at 18 months after treatment application. Samples were frozen immediately after collection, transported to the laboratory and oven-dried at 80 ºC until constant weights were achieved. Samples were then ground and 1.6 mg of dry material was loaded into tin capsules and placed in 99-well microtiter plates (Elemental Microanalysis Ltd; Okehampton, UK). Samples were sent to the Stable Isotope Facility at University of California, Davis, for isotopic analyses. Analyses were conducted using a continuous

flow combustion mass spectrometer. The isotopic composition was expressed relative to the PeeDee Belemnite (PDB) carbonate standard (Peterson and Fry, 1987).

Soil samples were taken within 0.5 m around the trunk for microbial activity analysis. A sample about 25 mm diameter and 100 mm long was collected using a soil probe (AMS Inc. American Fall, ID) before the initial treatment applications and four months before subsequent carbohydrates applications. Samples were analyzed in a lab to measure soil respiration as CO_2 evolved following the methodology described in Chapter II (Anderson, 1982). The results helped to investigate how multiple applications of carbohydrates might affect microbial activity and the lasting effect of the applications. One year after the first application, soil and leaves of trees from controls and 120 g·L^{-1} applications were collected and sent to A&L Plains Agricultural laboratories, Inc. at Lubbock, TX, for nutrient analysis.

The experimental design was completely randomized with a factorial structure augmented with the control (Lentner and Bishop, 1986) and using time in the model. Sum of squares (SS) and degrees of freedom (df) for carbohydrates and the interactions involving this factor as well as for the error term were modified in the ANOVA table of the factorial 4×3. Values (SS and df) from the data analysis as a factorial 3×3 (without the control) were used for carbohydrates and the interactions, while values (SS and df) from the analysis as a complete randomized design (10 treatments) were used for the error term. The results were analyzed using the procedure GLM with SPSS v.13. In the case of nutrient results and carbon isotope ratio (δ^{13}C), data was analyzed as a complete

randomize design and Dunnett's one tailed t-test was used when significant treatment effects were found ($P<0.05$).

Results and Discussion

Trunk growth was not significantly ($P>0.05$) affected by soil drench applications with glucose, starch and 50:50 mixtures at 40, 80, or 120 g·L^{-1} (data not shown). Previous research showed that applications of other sugars such as galactose and rhamnose did not have significant effects on tree growth (Percival or Fraser, 2005). However, sucrose, fructose, and glucose applied at 50 or 70 g·L^{-1} resulted in increased shoot growth in *Betula pendula* Roth (Percival and Fraser, 2005). The main difference with research showing significant increases in growth was associated with plant size (< 1 m). It seems that smaller plants are affected by carbohydrate concentrations similar to those used in this experiment.

Growth can be negatively affected by carbohydrate applications to the soil because soil microbes can compete and sequester nitrogen in the soil (Tate, 2000). In species such as *Festuca vivipara* (L.) Sm., carbohydrate additions within a dose of 12 or 25 g·L^{-1} reduced plant growth (Schmidt et al., 1997a). Also, lower concentrations of carbohydrates are utilized by microbes before they can have an impact on tree growth (Dassonville et al., 2004) while higher concentrations can have an indirect effect on growth (Schmidt et al., 2000). Even when the highest carbohydrate concentrations used were close to the total average glucose content in similar trees (assuming 40 Kg tree biomass) (McLaughlin et al., 1980), soil microorganisms or lack of root uptake can diminish the potential effect of carbohydrates on tree growth.

The use of digital photographs can provide a more sensitive technique for monitoring canopy growth, mainly in species with a decurrent growth habit such as live oak. In this study, the vertical canopy density revealed no significant differences among treatments ($P>0.05$). Results indicate that the concentrations used in the experiment failed to increase canopy growth, although it is possible that the results were affected by competition from canopies of adjacent trees at the nursery. Regardless of the lack of significant differences in this research, the use of this technique was useful measuring canopy growth through time and is still recommended for future research on trees with a decurrent form.

For root growth, the high variability among data did not allow for detection of significant differences in any of the measured variables (length, diameter, and dry weight) (data not shown). The results could also be affected by the small amount of roots collected. Percival et al. (2004) indicated that soil injections with sucrose (> 50 g·L^{-1}) improved fine root growth of *B. pendula*. In the same experiment, the authors also found an effect of sugar applications in *Quercus robur* L., but the results were unclear because the lowest (25 g·L^{-1}) and highest (70 g·L^{-1}) concentrations were not significantly different from each other while the middle (50 g·L^{-1}) concentration did result in differences.

Another objective of the experiment was to analyze the effect on tree vitality. Chlorophyll fluorescence, among other techniques, has been used to estimate tree vitality. Chlorophyll fluorescence can provide information about a plant's ability to tolerate environmental stresses (Maxwell and Johnson, 2000), and some parameters,

such as Fv/Fm, have been used to estimate plant vitality (Percival and Sheriffs, 2002).

Chlorophyll fluorescence values have also been used to detect differences within the

same species when using carbohydrate applications as soil drenches (Percival and

Fraser, 2005). Carbohydrate applications as soil drenches can directly or indirectly affect

tree growth in at least three different ways. First, sugar applications can increase the

carbohydrate interchange between the soil and the root system resulting in carbohydrate

uptake (Cheshire, 1979; Percival and Fraser, 2005). Another effect can be that sugar-

induced increases in microbial biomass intensify the mineralization of organic matter

making more nutrients available for trees resulting in a potential increase in plant growth

(Schmidt et al*.,* 1997a). The third effect is that microorganisms will sequester or

immobilize nutrients resulting in a reduction in plant growth (Schmidt et al*.,* 2000).

None of these effects was clearly detected in this experiment within the two-year study

period.

Table 4.1. Significance values from ANOVA for chlorophyll fluorescence (Fv/Fm), intensity of green color in the canopy, and glucose content in twigs of live oaks treated with three carbohydrate types (glucose, starch and a 50:50 mixture) at four concentrations (0, 40, 80, and 120 g·L^{-1}).

Source	Chlorophyll fluorescence (Fv/Fm)	Green intensity	Glucose content	Soil respiration
Concentration	0.160	0.037	0.018	0.046
Carbohydrate	0.025	0.070	0.146	0.062
Time	0.001	0.001	0.001	0.005
Carb. * conc.	0.379	0.342	0.393	0.027
Carb. * time	0.021	0.987	0.644	0.998
Conc. * time	0.033	0.936	0.786	0.950
Carb * Conc. * time	0.355	0.999	0.997	0.949
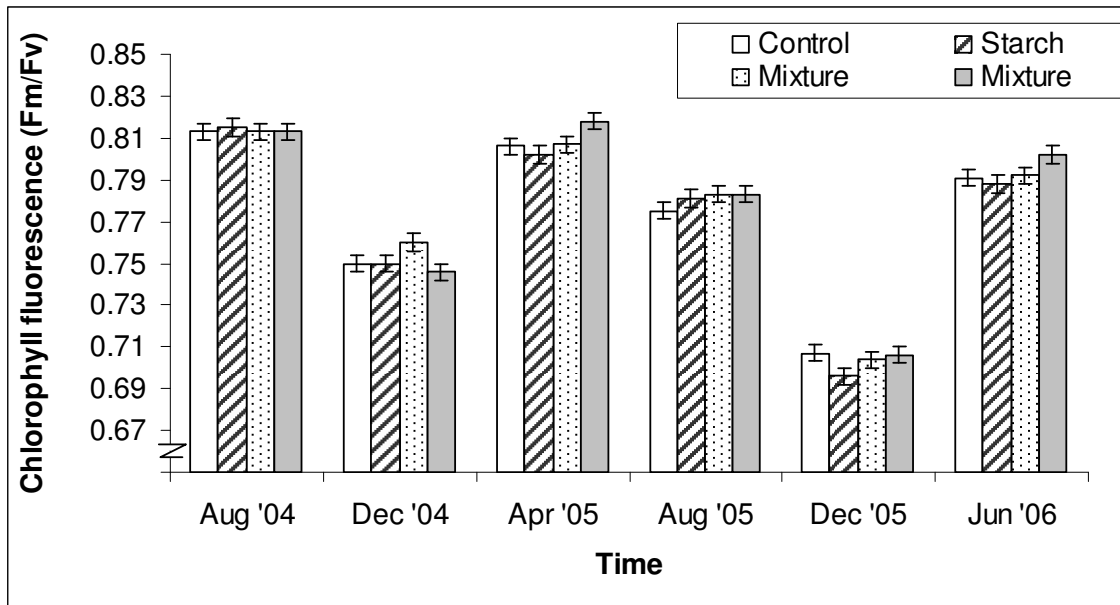
Fig. 4.1. Overall seasonal effects of exogenous applications of carbohydrates (glucose, starch, and a 50:50 mixture) on chlorophyll fluorescence (Fv/Fm) of live oaks. (Bars show the standard error).

Average chlorophyll fluorescence Fv/Fm values varied among different carbohydrates and concentrations throughout the experiment (Fig. 4.1). At the beginning of the experiment, Fv/Fm values were similar among treatments, but values for the mixture treatment were higher in the third and final assessment date (Fig. 4.1). When assessing concentrations, results showed that after a year of sugar applications the lowest and middle concentrations (40 and 80 g·L^{-1} respectively) had higher Fv/Fm values compared with the control (Fig. 4.2). Unfortunately, the results did not demonstrate a clear trend, so a particular carbohydrate cannot be recommended for improving stress tolerance or tree vitality for live oaks. The intensity of the green color of the canopy measured from digital photographs also showed differences between the types of carbohydrates used and the control. The overall mean indicates that treated trees

exhibited a greener intensity compared with the control (data not shown). Kent et al. (2004) found that leaf chlorophyll concentrations were not recommended as a useful stress indicator in *Quercus virginiana* P. Mill. var. *germinata* (Small) Sarg.
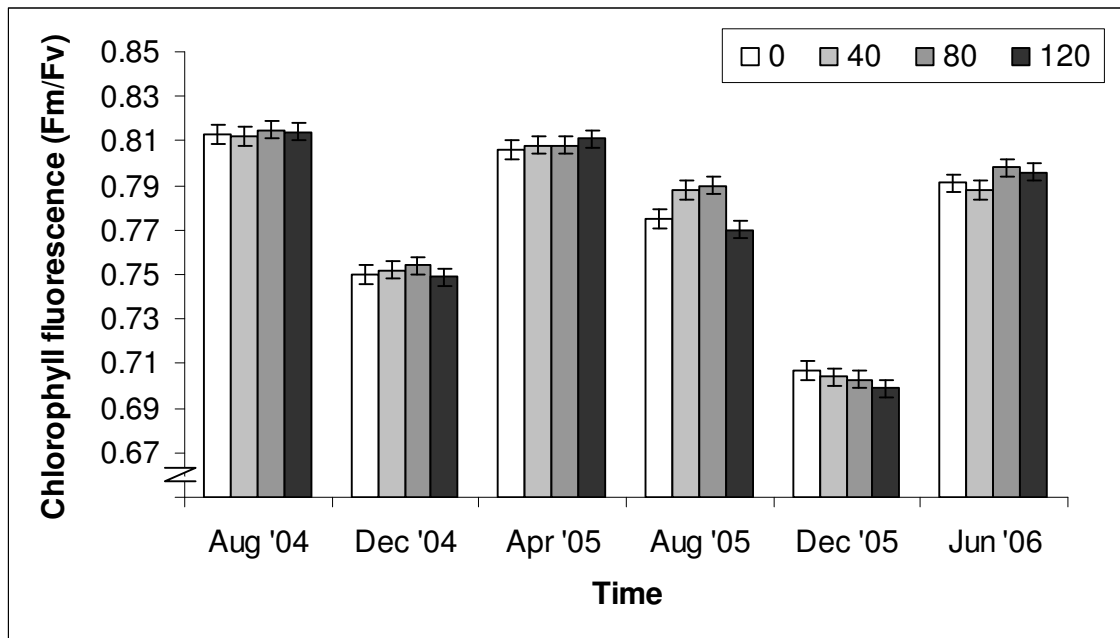
Fig. 4.2. Overall effects of carbohydrate concentrations (0, 40, 80, and 120 g·L^{-1}) on chlorophyll fluorescence in live oaks over time. (Bars indicate the standard error).

Net carbon assimilation was 8.95 µmol m^{-2} s^{-1} ± 2.18 µmol m^{-2} s^{-1} SD during the experiment. The overall mean was similar to values reported in previous research for this species (Tognetti and Johnson, 1999), but results from this experiment did not reveal differences ($P>0.05$) among types or concentrations of carbohydrates (data not shown).

While there were no significant differences in starch levels in twigs among the treatments, overall glucose content in the twigs was affected by the treatments (Table

4.1). Results indicate that applications with higher carbohydrate concentrations affected the concentration of glucose in twigs (Fig. 4.3). The results do not clearly indicate that this effect was caused by an uptake of glucose from the root system or by another indirect effect. The use of twigs for monitoring treatment effects was used because they are present in all seasons, can be easily collected, and have lower variability when measuring carbohydrate concentrations (shown in preliminary results). In order to understand the effect of treatments on energy storage organs in trees, carbohydrate content in root tissues was also analyzed after several carbohydrate applications. However, after 16 months glucose and starch content in root tissues did not show differences among treatments (data not shown).

Carbon isotope ratio (δ^{13}C) has been used as a way to evaluate stress tolerant conditions in trees, but it has also been used to distinguish between C_3 and C_4 plants (Eleki et al., 2005). In this study, this technique was used to determine the potential uptake of carbohydrates. Considering that live oaks are C_3 plants and that the applied carbohydrates were from C_4 plants, a difference in the δ^{13}C signature of tissues from treated trees versus the control could indicate the uptake of C_4 plant-derived carbohydrates from exogenous sources. Carbon isotope ratio results were within the range of δ^{13}C values for C_3 plants between -20 to -34 ‰ (Pate and Dawson, 1999) and close to the values reported for *Quercus virginiana* P. Mill. var. *fusiformis* (Small) Sarg. (Jessup et al., 2003). The δ^{13}C results did not show differences among the control and samples from trees treated with glucose or starch at 40 or 120 g·L^{-1} (Table 4.2). Assessment of δ^{13}C signatures have also been used to detect tolerance to stress

conditions and to determine the types of plants in past ecosystems (Fotelli et al., 2003; Stock et al., 2004). However, the results did not show any evidence that potential sugar uptake can alter the carbon isotope ratio considering that exogenous carbohydrates were from C_4 plants with different $\delta^{13}C$ signature. Since only twig samples were used, it is possible that exogenous carbohydrates were translocated or stored in other organs throughout the tree. These results failed to precisely reveal whether or not the exogenous carbohydrates were actually taken up by the trees.
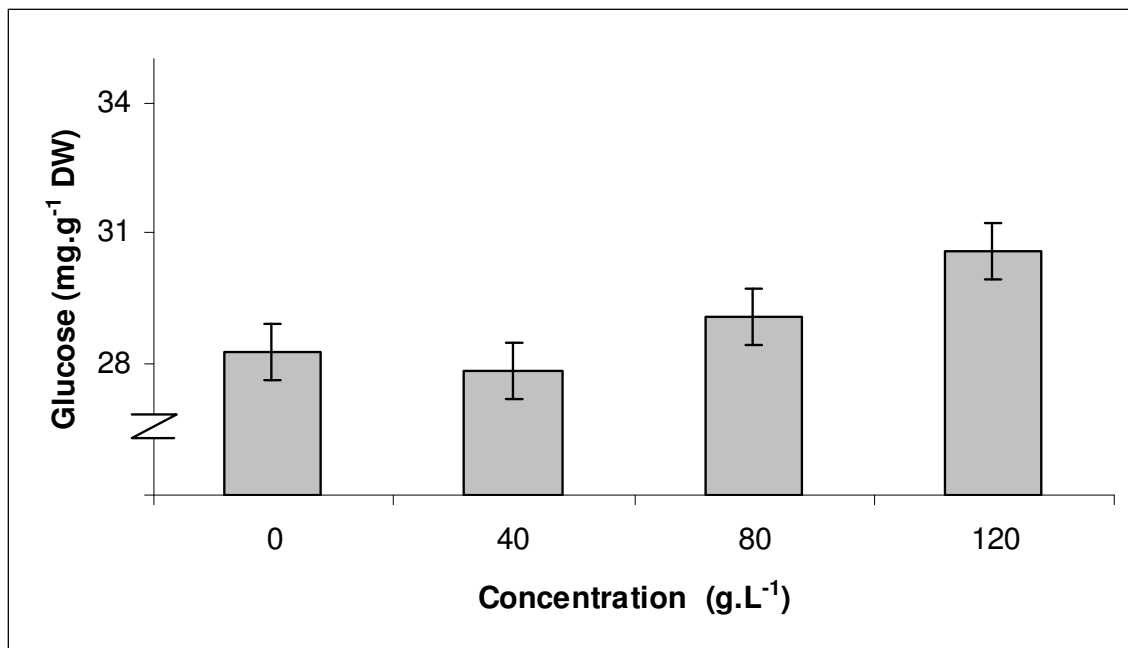
Fig. 4.3. Overall effects of exogenous applications of carbohydrates (glucose, starch, and a 50:50 mixture) at four concentrations on glucose content of twigs in live oaks. (Bars indicate standard error).

Table 4.2. δ^{13}C signatures (± standard deviation) in twigs and roots of live oaks treated with glucose and starch at 0, 40 and 120 g·L^{-1} after 18 months.

Treatment	δ^{13}C PDB (‰)	
	Twigs	**Roots**
Control	-28.41 ±0.55	-24.40 ±0.23
Glucose 40	-27.57 ±0.59	-26.50 ±0.71
Glucose 120	-28.47 ±0.65	-27.10 ±0.80
Starch 40	-28.31 ±0.47	-26.50 ±0.53
Starch 120	-28.11 ±0.49	-26.20 ±0.56

In order to monitor the effects of carbohydrate applications on soil microbial activity during the experiment, soil respiration was determined four months after treatment, which revealed significant differences (Fig. 4.4). The increase in microbial activity was similar to previous research using similar carbohydrates and concentrations (Illeris and Jonasson, 1999; Jonasson et al., 1996). Even though there was a significant increase in soil respiration after starch application, no significant increase in growth or vitality was detected. In addition, nutrient analysis of soil and leaves did not show differences between the control and the treated samples after a year of being treated (data not shown).
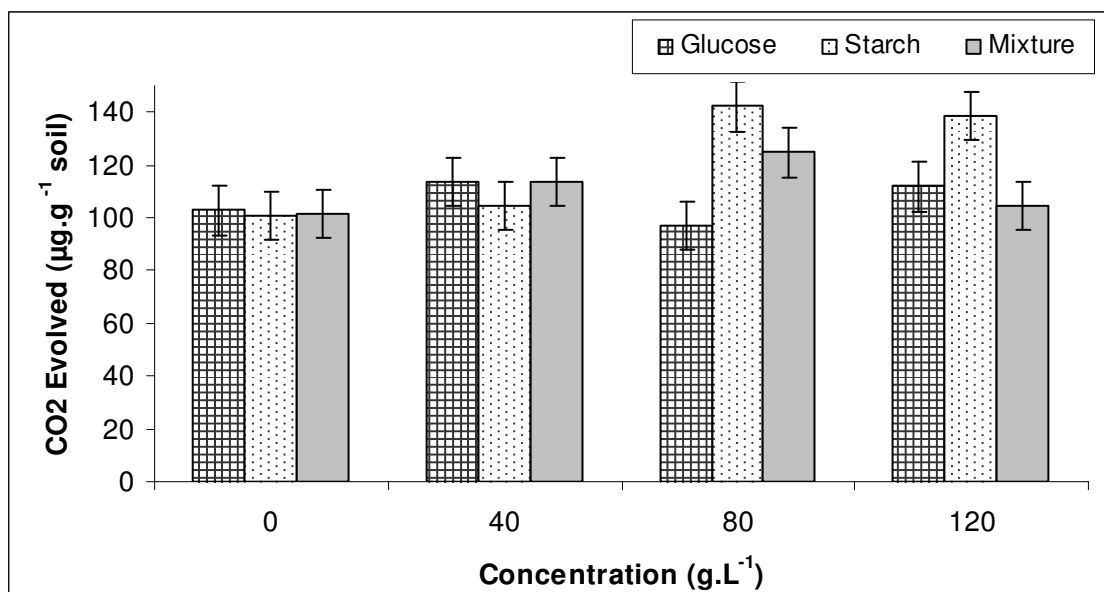
Fig. 4.4. Overall effects of carbohydrates (glucose, starch, and a 50:50 mixture) at different concentrations (0, 40, 80, and 120 g·L^{-1}) on soil respiration (µg CO$_2$·g^{-1}) four months after applications. (Bars show standard error).

Results showed that exogenous applications of carbohydrates (glucose, starch, and a 50:50 mixture) had no significant impact on growth. Even when higher concentrations showed greener intensity and some effect on chlorophyll fluorescence, the differences were low and did not reveal a clear trend. The highest concentration of starch (120 g·L^{-1}) had a significant impact on microbial activity four months after being applied, but these changes did not produce a significant effect on growth or a considerable effect on tree vitality during the experiment.

This study investigated the impact of applying high concentrations of carbohydrate to trees, and the results demonstrated that carbohydrate applications were not harmful to trees. Consequently, higher applications in future experiments may

produce better results. In addition, this information is useful for future studies using other applications techniques such as tree injections or the application of carbohydrates on stressed and declining trees.

CHAPTER V

EFFECTS OF CARBOHYDRATE TRUNK INJECTIONS ON GROWTH AND

VITALITY OF LIVE OAKS

Photosynthesis in leaves and other chlorophyll-containing tissues produces carbohydrates, which are converted to energy by respiration (Pallardy, 2008). Carbohydrates can be used in situ, transported to organs where they are needed, or stored for future use (Taiz and Zeiger, 2006). Trees allocate carbohydrates for maintenance, reproduction, growth, and/or defense based on environmental factors and growth stage (Pallardy, 2008). Research has shown that tree growth and vitality depend on carbohydrate content in tree organs (Kosola et al., 2001; Wargo et al., 1972). When trees are affected by stress-inducing factors, carbohydrate levels can be decreased or depleted, which can have negative repercussions on growth and vitality (Gregory and Wargo, 1985). Urban trees are commonly subjected to stressful conditions that can negatively impact tree vitality. Previous research has shown that improvement in tree vitality is directly affected by the energy level in trees (Carroll et al., 1983; Percival and Smiley, 2002; Wargo, 1975). The use of inexpensive, non-toxic, and environmentally-friendly products such as sugars could help improve growth and vitality of trees (Percival et al., 2004).

Applications of carbohydrate solutions directly to tree root systems have been suggested as a way to improve root growth and vitality (Percival and Fraser, 2005; Percival et al., 2004). Although soil drenches of carbohydrate solutions are probably the easiest way to apply carbohydrates to trees, several factors may impact the effectiveness

of this method. For example, soil microorganisms can quickly utilize carbohydrates once they are applied to the soil (Schmidt et al., 2000). Irrigation or rainfall can reduce the effect of applied carbohydrates by leaching the carbohydrates from the soil before root uptake occurs. In addition, carbohydrate uptake by roots under field conditions is difficult to assess. Therefore, other methods such as trunk injections can be more appropriate for the applications of exogenous carbohydrates (Iglesias et al., 2001).

Trunk injection methods have been useful for introducing various compounds into trees. The most common types of injections on trees include bark banding, trunk infusion, and pressurized trunk injections (Sachs et al., 1977; Sanchez and Fernandez, 2004). Trunk injections are classified as micro- or macroinjections according to the amount of material injected (Costonis, 1981). Macroinfusion is a trunk injection system that has been used for applying higher amounts of solutions into trees without producing considerable damage (Appel, 2001; Eggers et al., 2005). This method makes it easier to control the amount of sugars injected when using higher volumes of solution compared with other microinjection systems.

The increase of plant carbohydrate levels as a result of injections can have an effect on growth and vitality (Abdin et al., 1998; Iglesias et al., 2001). In the case of sucrose microinjections in fruit trees, research has shown little effect on fruiting (Iglesias et al., 2003; Iglesias et al., 2001). The main reason for the results was the low amount of solution injected into the tree by the microinjection system. Anecdotal reports of sucrose macroinjections in the trunk of a large, historic live oak showed some apparent vitality

improvement after being treated (Giedraitis, 1990). Unfortunately, there are no scientific research studies on macroinjections of carbohydrates in urban trees.

Tree growth is one of the most common indicators used for studying the effect of environmental factors or treatments on growth and vitality (Dobbertin, 2005). The application of carbohydrates through trunk injections may increase the energy pool and generate greater growth rates (Percival et al., 2004). Injected solutions may move up through the xylem, or they may be stored or translocated to storing tissues (Sachs et al., 1977; Tattar and Tattar, 1999). Considering that exogenous carbohydrates can be translocated to different parts of the tree, variables in addition to growth should be measured to assess tree vitality and effects caused by carbohydrate supplementation.

Various tools have been suggested for determining tree vitality in the field. Chlorophyll fluorescence parameters such as Fv/Fm are suggested as one measurement of tree stress tolerance and tree vitality (Percival and Sheriffs, 2002). The parameter Fv/Fm is often used for measuring the photochemical efficiency of the photosystem II, which indicates the level energy absorbed by chlorophyll and damage by excess light (Maxwell and Johnson, 2000). An advantage of using chlorophyll fluorescence measurements is the ease and speed of collecting data using a portable fluorescence spectrometer.

Photosynthesis measurements are also important for providing additional information about tree vitality and treatment effects. Carbohydrate injections could affect photosynthetic processes considering that sugars and water are incorporated into the vascular system and possibly moved up through the canopy (Percival and Fraser,

2005; Tattar and Tattar, 1999). The effect may be less evident if the sugars are mainly translocated to storage organs such as trunk or roots. Therefore, tracking carbohydrate content in twigs and roots can help to determine the effect of exogenous applications.

Recently, carbon isotope ratio (δ^{13}C) has been used to determine stress tolerance in trees (Pate and Dawson, 1999). Because δ^{13}C signatures have been used to identify C_3 and C_4 plants (Fotelli et al., 2003), this variable could give information about the translocation of exogenous sugars to different parts of the tree. When carbohydrates from C_4 plants (e.g. *Zea mays* L.) are injected into C_3 plants (e.g. *Quercus virginiana*), locating and quantifying exogenous carbohydrate can be done by comparing carbon isotope ratios between treated and non-injected trees. This information would be useful for determining the fate and impact of carbohydrate supplementation in trees.

Information about the effects of introducing exogenous carbohydrates as a source of energy might provide arborists with a technique to improve the health of urban trees. The main goals of this investigation were to study the effects of trunk injections of carbohydrates on growth and vitality of live oak and to assess the potential for tracing exogenous carbohydrates using carbon isotope ratios.

Materials and Methods

Thirty-six established, field-grown live oaks (*Q. virginiana*) (16-20 cm dbh) grown under similar conditions were used. Similar trees were selected from a group of unirrigated trees planted on a 6-m spacing in an urban forest near College Station, TX in Burleson County (30º33'14.71"N, 36º25'33.61"W). Trees were growing in a Weswood silty clay loam soil. The site has an annual mean temperature of 20.3 ºC (14.2 ºC

minimum, and 26.3 ℃ maximum) and annual precipitation varies between 762 and 1016 mm.

Trunk injections using glucose, sucrose, or a 50:50 mixture of glucose and sucrose by weight in three different concentrations (40, 80, and 120 $g \cdot L^{-1}$) were used. Nine trees served as a water-only control, and three trees were injected for each concentration and type of carbohydrate. The concentrations were determined according to previous research on carbohydrate applications on plants (Abdin et al., 1998; Iglesias et al., 2003; McLaughlin et al., 1980). Approximately 10 L of solution were injected into the buttress roots using injection protocols established for injecting trees for oak wilt (Appel, 2001; Eggers et al., 2005). Trees were injected during January 2005 and again in January 2006.

Trunk diameters were measured at 30 cm above ground using a diameter tape (Forestry Suppliers Inc.; Jackson, MS) and recorded three times during the year throughout the experiment. To avoid possible effects of initial size among trees, a growth index was calculated per year by dividing the absolute increment in a year by the first measure (Arnold et al., 2007). Growth index values were used for the statistical analysis.

Four soil holes (15 cm deep x 6 cm diameter) were dug 1.5 m from the trunk and refilled with sandy loam soil to evaluate root growth. Core samples were extracted using a core sampler one year after treatment application. An herbicide (glyphosate) was applied periodically throughout the experiment to control weeds. Root lengths and average root diameters were measured using the Winrhizo software® (Regent

Instruments Inc., Québec, Canada). Soil samples were collected annually in the same location to evaluate new root growth among treatments.

Twig samples were collected three times each year for carbohydrate analysis. To avoid differences between trees, samples were taken from the lowest third of the canopy in all trees. Glucose and starch content were determined for each sample using Sigma® GAGO-20 reagents (Sigma®, St. Louis, MO). Glucose was extracted from tissue in methanol:chloroform:water (MCW, 12:5:3:, v/v/v) solution after centrifugation at 2800 rpm. A 0.5-mL aliquot of the extract and the glucose standards were mixed with 5 mL of anthrone reagent (Jaenicke and Thiong'o, 1999). Starch content was determined in the remaining pellet using the enzyme amyloglucosidase, which converts starch to glucose. Absorbance of samples and standards was read within 30 minutes with a spectrophotometer (Spectronic 20, Baush & Lomb, Rochester, NY) set at 625 nm for glucose and 540 nm for starch (Haissig and Dickson, 1979; Renaud and Mauffette, 1991).

Net carbon assimilation was measured in each treatment using a portable photosynthesis system LI-6200 (Li-Cor®, Lincoln, NE). Carbon assimilation was measured in the morning on sunny days on the southern side of the canopy. Three leaves from the lowest third of the canopy were selected. To avoid differences between measurements due to time, only three trees per treatment were measured.

Chlorophyll fluorescence was measured using a HandyPEA® portable fluorescence spectrometer (Hansatech Instruments Ltd, King's Lynn, UK). Ten leaves from the lower two-thirds of the canopy were adapted to darkness for 25 minutes. After

the darkness period, the fluorescence response was induced by a red light of 600 W.m^{-2} intensity provided by an array of 6 light-emitting diodes, with a data acquisition rate of 10 µs for the first 2 ms and 12-bit resolution (Percival and Fraser, 2001; Percival and Fraser, 2005). Chlorophyll fluorescence data was taken at January, April, August, 2005, and January, April, August 2006, and January 2007.

The translocation of carbohydrates was evaluated by determining carbon isotope compositions. Twigs and roots samples were collected 12 months after being first treated from controls, glucose (40 and 120 g·L^{-1}), and sucrose (40 and 120 g·L^{-1}) treatments. Samples were submitted for analysis to the Stable Isotope Facility at University of California, Davis. The isotope composition was expressed to PeeDee Belemnite (PDB) carbonate standard (Peterson and Fry, 1987).

The experimental design was completely randomized using three trees per treatment. The data was analyzed using an augmented factorial structure considering time in the model (Lentner and Bishop, 1986). Because carbon isotope ratio was determined only for some treatments, data were analyzed using a complete randomized design. When the main factors were significant ($P<0.05$), mean comparisons were calculated using Dunnet's test comparing the treatments with the control. The results were analyzed using the SPSS v.13 software.

Results and Discussion

Trunk growth indices revealed a significant difference ($P<0.05$) among the overall mean of carbohydrates, but not for concentrations (Table 5.1). This might suggest that either the concentrations were insufficient to affect tree growth or that

sugars were used for processes other than growth. Because trees were not under visibly stressful conditions, the trees may have used exogenous carbohydrates for other functions such as storage, defense, or reproduction (Pallardy, 2008). Iglesias et al. (2001) found that *Citrus unshiu* (Mak.) Marc., cv. Okitsu enhance fruit set by 10% when supplemented with sucrose. Early studies showed how albino corn was able to survive and produce inflorescences with supplementation of sucrose through the cut ends of leaves (Spoehr, 1942).

Table 5.1. Significance values from the ANOVA table for growth index, glucose in twigs, starch in roots, and chlorophyll fluorescence Fv/Fm for live oaks injected with three sugars (glucose, sucrose, and a 50:50 mixture) and four concentrations (0, 40, 80, and 120 $g \cdot L^{-1}$).

	Diameter growth index	Glucose in twigs	Starch in roots	Chlorophyll fluorescence Fv/Fm
Concentration	0.159	0.036	0.001	0.001
Carbohydrate	0.049	0.941	0.881	0.104
Time	0.001	0.001	0.001	0.001
Conc. x carbohydrate	0.532	0.404	0.152	0.216
Conc. X time	0.334	0.469	0.002	0.064
Carbohydrate x time	0.133	0.531	0.627	0.141
Conc. X carb. x time	0.160	0.974	0.209	0.767

The results also indicate that the 50:50 mixture of glucose and sucrose resulted in a small but significant increase in the overall growth index mean as compared to sucrose and glucose alone (Fig. 5.1). Sucrose is the type of sugar translocated by phloem while glucose is a simple carbohydrate product of photosynthesis and is the base unit of storage carbohydrate (Taiz and Seizer, 2006). The supplementation with different sugars

through trunk injections in live oaks might have an added effect and help trees to utilize carbohydrates better to increase growth. In other studies, growth was also stimulated in annual plants such as soybean (*Glycine max* (L.) Merr.) and corn (*Z. mays*) when they were treated with sucrose injections at 300 g·L^{-1} and compared with the control (Abdin et al., 1998; Zhou et al., 1997). It seems that the amount injected and size of plants played an important role in the potential effect of carbohydrates injected. In addition, research indicates that carbohydrates such as sucrose and glucose can affect sugar sensing systems that initiate changes in gene expression, which can cause an effect on plant growth (Koch, 1996).

Fig. 5.1. Overall diameter growth indices (cm·cm^{-1}) of live oaks injected with three different types of sugars (glucose, sucrose, and a 50:50 mixture). Bars show standard error.

When the absolute values for trunk increment were analyzed, similar results were found among types of sugars, but statistical differences were also found among concentrations (P=0.016). The highest concentrations (120 g·L^{-1}), regardless of the type of sugar, demonstrated higher trunk increments compared with the control (Fig. 5.2). Trunk increments were increased with higher concentrations of all sugars tested with the greatest increase found in the 50:50 mixture. However, these differences among concentrations were probably affected by other factors because results were not confirmed when growth indices were analyzed. Results with sucrose injections in soybean plants also showed a direct relationship between growth and sucrose concentration (Abdin et al., 1998). Research has shown that the injected volume of sugars can be affected by the carbohydrate concentration in small plants (Zhou and Smith, 1996), but we did not experience this problem using the macroinfusion technique in live oaks.
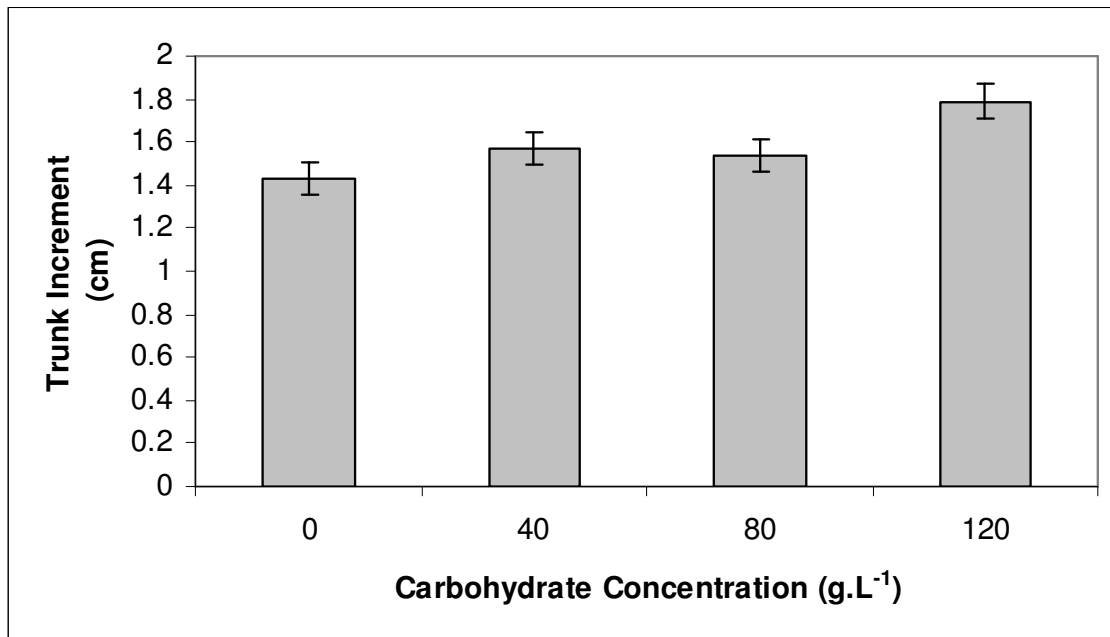
Fig. 5.2. Overall mean of trunk diameter growth (cm) of live oaks injected with four different carbohydrate concentrations (0, 40, 80, and 120 g·L^{-1}). Bars show standard error.

Results of root growth did not show significant differences ($P>0.05$) among sugars or concentrations. It seems that the effect of injections was higher in the aboveground portions of the tree. However, the determination of root growth was based on sampling a small portion of fine roots (four samples per tree), which might be the reason for the lack of significant differences among the results. In addition, data showed high variation among samples (data not shown). Previous research has shown an increase in fine roots as a result of exogenous applications of sucrose which apparently caused suppression in photosynthesis and carbon remobilization in favor of enhancing root development (Abdin et al., 1998; Percival et al., 2004).

There were no significance differences ($P>0.05$) found in net carbon assimilation among different sugars or concentrations during the two year period. However, the data showed high variation, which might have affected the analysis. Also, trunk injections were performed during the dormant season with old leaves before leaf emergence, which could have reduced the potential effect on photosynthesis. In soybean plants, the supplementation with sucrose by injections suppressed photosynthesis (Abdin et al., 1998); however, the effects in this study could have been caused by a response to the injections themselves.

Starch content in twigs and glucose content in roots did not show significant differences, but glucose content in twigs and starch in roots showed significant differences ($P<0.05$) among overall mean of concentrations (Table 5.1). The glucose content in twigs and the starch in roots showed an increase in treatments treated with the highest concentrations (120 g·L^{-1}). This type of result was anticipated due to the potential translocation of exogenous carbohydrates either upwards or downwards as a result of the supplementation of sucrose and glucose by the injections (Tattar and Tattar, 1999). Prior research showed how ^{14}C sucrose infused into sorghum (*Sorghum bicolor* (L.) Moench) plants via a pulse application can move upwards through the xylem (Tarpley et al., 1994). Corn plants (*Z. mays*) formed abundant starch when they were laced in solutions of glucose or sucrose (Spoehr, 1942). Similar results were also found in plants of Satsuma mandarin (*C. inshui* cv. Okitsu) injected with sucrose where fine roots showed high levels of starch (Iglesias et al., 2001). The differences in this study were more evident in root starch where the highest concentrations (120 g·L^{-1}) showed

higher differences compared with the control (Fig. 5.3B). Exogenous carbohydrates could have been either stored or translocated to the roots (Tattar and Tattar, 1999). Higher carbohydrate concentration in other organs like roots and fruits has been reported for Satsuma mandarin when carbohydrates were injected in the trunk using sucrose (Iglesias et al., 2003).

Results with chlorophyll fluorescence Fv/Fm showed significant differences ($P<0.001$) among overall concentrations. Trunk injections using carbohydrates increased the chlorophyll fluorescence index (Fig. 5.4). Abdin et al. (1998) found that a concentration of 300 g·L^{-1} negatively affected the Fv/Fm ratio while 150 g·L^{-1} increase Fv/Fm values in soybean plants. Considering the results on trunk diameter growth, it was expected that the mixture treatment had higher chlorophyll fluorescence values, which could have been a result of an improvement in growth. However, exogenous carbohydrates could have been used as reserves and used later in generating trunk diameter increases, or injections might have affected photosynthesis even though results in this experiment failed to detect differences among treated trees.

Fig. 5.3. Overall effects of carbohydrates (glucose, sucrose, and a 50:50 mixture) supplemented by trunk injections at four concentrations (0, 40, 80, and 120 g·L^{-1}) on glucose content from twigs (A) and starch content from roots (B) of live oaks. Bars show standard errors.

Chlorophyll fluorescence measures the photochemical efficiency of photosystem II (Maxwell and Johnson, 2000) and is used as a non destructive diagnostic system to measure plant vitality and early diagnosis of stress (Percival and Boyle, 2005; Percival,

2004; Percival and Sheriffs, 2002). The supplementation of carbohydrates via trunk injections showed an increase in the overall Fv/Fm values so that exogenous carbohydrates applications can be suggested as a way to improve vitality. In addition, chlorophyll fluorescence showed a similar trend with other variables used to determine tree health such as trunk growth, glucose content in twigs, and starch content on roots, (Dobbertin, 2005; Gregory and Wargo, 1985; Wargo et al., 2002). The results indicate that carbohydrate content may be more closely associated with tree vitality than growth since the results among those variables showed a similar trend.

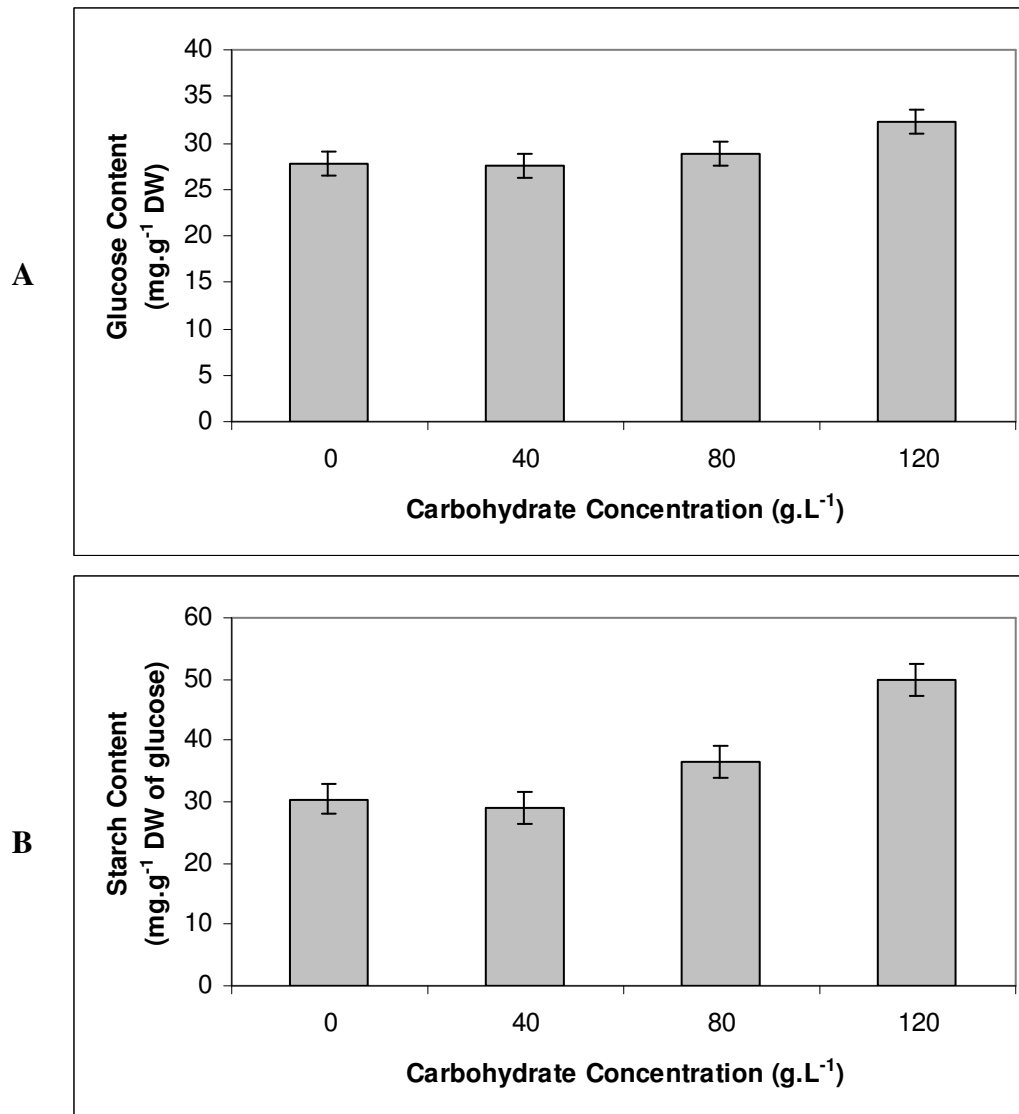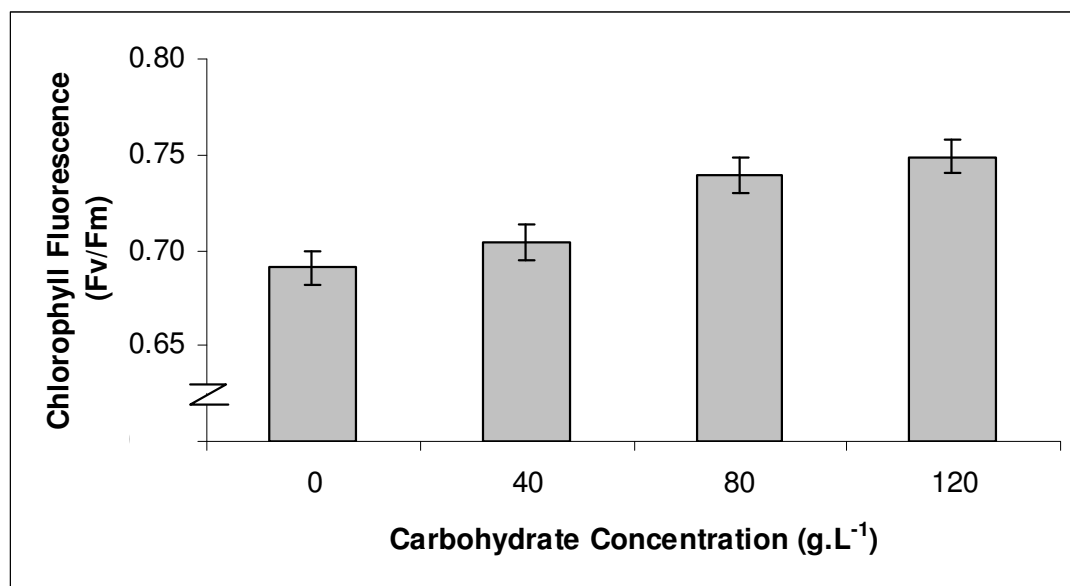**Fig. 5.4. Overall effects of carbohydrates (glucose, sucrose, and a 50:50 mixture) supplemented by trunk injections at four concentrations (0, 40, 80, and 120 g·L^{-1}) on chlorophyll fluorescence (Fv/Fm) on live oaks. Bars show standard error.**

The carbon isotopic ratios (δ^{13}C) did not demonstrate significant differences among root samples, but did show significant differences ($P<0.05$) when comparing

some treatments against the control (Table 5.2). Although the use of carbon isotopic ratio was mainly used to identify the presence of exogenous carbohydrates used from C_4 plants, the results did not show a clear trend that supported the presence of exogenous carbohydrate sources. However, values of $\delta^{13}C$ in twigs from trees injected with 120 g·L^{-1} of sucrose were significantly different from the control possibly indicating a small presence of exogenous carbohydrates (Table 5.2).

Table 5.2. Carbon isotopic ratio ($\delta^{13}C$) in twigs and roots of live oaks treated with glucose and sucrose at 0, 40 and 120 g·L^{-1}.

Treatment	Roots	Twigs
Control	-26.823	-30.530
Glucose 40	-26.437	-30.430
Glucose 120	-26.823	-30.037
Sucrose 40	-26.697	-29.973
Sucrose 120	-26.697	-29.157 *

* significantly different ($P<0.05$) using Dunnet's test comparing all the groups against the control.

Previous studies showed that basal area growth and carbon isotopic ratio are related (Garcia et al., 2004; Walcroft et al., 1997). When mean values from twigs and trunk increments were compared, it was found that sucrose showed the lowest $\delta^{13}C$ values and the highest growth rates. The lack of significant differences between glucose and the control might indicate that exogenous sugar was translocated to other areas that were not sampled or that the amount of sugar injected was low compared with the amount naturally produced and used by plants.

Results from this experiment showed how annual trunk injections of carbohydrates can improve growth and vitality in live oaks. No visual or physiological

damage was detected as a result of carbohydrate injections during the time of the experiment. Previous research showed that carbohydrates can help combat the effect of stress conditions, such as defoliations (Iglesias et al., 2003). Based on the results of this study, future research on the effects of carbohydrate injections in trees subjected to stressful conditions should be conducted where the impact on tree performance may be more pronounced.

CHAPTER VI

A COMPARISON OF FIELD TECHNIQUES TO EVALUATE TREE VITALITY OF

LIVE OAK

Urban trees are impacted by adverse man-induced or naturally occurring stressors that can cause a loss of vitality. Vitality has been considered as a tree's ability to deal effectively with stress (Lilly, 2001). Carbohydrates are the main source of energy for plants so that their content in trees has been used as an indicator of tree vitality (Kosola et al., 2001; Wargo, 1975). Carbohydrates are produced in mature leaves and then translocated through the phloem to areas where they are needed or stored (Taiz and Zeiger, 2006). Branches, trunk and roots can store carbohydrates and are involved in translocation of carbohydrates through the phloem between sources and sinks (Pallardy, 2008). The impact of adverse environmental conditions can affect tree vitality by altering the carbohydrate balance in urban trees (Dunn and Rowland, 1986). Although various techniques have been suggested to measure tree vitality, the procedures have not been well studied in urban trees. Arborists require reliable and practical techniques for detecting tree vitality in the field.

Analysis of carbohydrate content in several tissues has been used as an indicator of tree vitality (Kosola et al., 2001; McCullough and Wagner, 1987; Wargo, 1975; Wargo et al., 1972). The content of starch and glucose in tree organs may indicate a tree's ability to withstand stressful conditions (Bardanoux et al., 2003; Tschaplinski and Blake, 1994). Unfortunately, the analysis of carbohydrate content requires laboratory

protocols, which makes this technique unfeasible as a practical option in the field (Haissing ad Dickson, 1979). Alternative practical methods have been suggested for evaluating carbohydrate content such as staining tissues with Lugol's solution for starch content (Kolosa et al., 2001; Wargo, 1979). Additionally, the use of portable carbohydrate meters such as refractometers can offer another practical option for determining sugar content from tree tissues without complicated laboratory protocols (Waes et al., 1998).

Another important tool that has been suggested for determining vitality is the use of chlorophyll fluorescence spectrometers (Maxwell and Johnson, 2000). Chlorophyll plays an important role in the conversion of light energy through photosynthesis to chemical energy (Taiz and Zeiger, 2006). Chlorophyll fluorescence Fv/Fm indicates the photochemical efficiency of the photosystem II, and the parameters are useful for assessing the overall ability of trees to produce sufficient carbohydrates necessary to face adverse conditions (Percival and Fraser, 2005). Chlorophyll fluorescence on leaves has also been suggested as a way to diagnose plant stress caused by negative environmental factors (Maxwell and Johnson, 2000; Percival, 2004). In addition, previous research has recommended the use of chlorophyll fluorescence parameters such as Fv/Fm for assessing tree vitality (Percival and Fraser, 2005; Percival and Sheriffs, 2002). The use of a portable fluorescence spectrometer can simplify the assessment of vitality, resulting in a rapid method for assessing trees in the field.

Measurement of electrical resistances in the cambial zone (phloem, cambium and bark) in the trunk has also been suggested as an easy technique for assessing tree vitality

(Blanchard et al., 1983; McCullough and Wagner, 1987). The use of electrical resistances within tree trunks has been used in the evaluation of physiological processes associated with stress conditions (Ostrofsky and Shortle, 1989). The readings are based on moisture and ion content of the cambial zone (Shigo and Shortle, 1985). Lower readings indicate a thicker cambial zone, which generally indicates higher vitality. In addition, resistance readings at the trunk may provide information about the growth potential, which is another measurement of vitality (Blanchard et al., 1983; Filip et al., 2002). The use of field ohmmeters such as the Shigometer® allow a practical way to measure electrical resistances and can be useful to corroborate the vitality of urban trees (Ostrofsky and Shortle, 1989).

Evaluating and contrasting carbohydrate content determined with laboratory analysis with proposed field techniques can help to qualify the applicability of field tools for determining vitality of urban trees. Although some tools for assessing tree vitality have already been tested and shown promising results in some studies in forest stands (McCullough and Wagner, 1987; Narog et al., 1997; Paysen et al., 2006), the tools need to be evaluated in other species under urban conditions to evaluate their feasibility for arborists. The goal of this paper is to compare laboratory analysis of carbohydrate content with practical field methods, such as Shigometer®, HandyPEA®, glucose meter, Lugol's solution, and refractometer, to evaluate the vitality of live oak.

Materials and Methods

Comparison of field and laboratory methods

Thirty-two live oaks (*Quercus virginiana*) exhibiting varying vitality conditions in an urban forest near College Station, TX at Burleson County (30º33'1.45"N, 96º25'35.66"W) were studied. The type of soil in the site was Ships clay described as very deep, nearly level, and moderately alkaline clay soil. The site presents an annual mean temperature of 20.3 ºC (14.2 ºC minimum, and 26.3 ºC maximum), and the annual average precipitation varies between 762 to 1016 mm. The trees were 15 cm diameter (dbh) and spaced 2 to 3 m apart. Woody samples were collected from buttress roots using an increment hammer (Haglof©; Langsele, Sweden) and processed for the determination of glucose and starch content in the laboratory using the Sigma® GAGO-20 reagents. Glucose was extracted from tissue in methanol:chloroform:water (MCW, 12:5:3, v/v/v) solution centrifuged at 2800 rpm. A 0.5 mL aliquot of the extract and the glucose standards were mixed with 5 mL of anthrone reagent (Jaenicke and Thiong'o, 1999). Starch content was determined in the remaining pellet using the enzyme amyloglucosidase, which converts starch to glucose. Absorbance of samples and standards was read within 30 minutes with a spectrophotometer (Spectronic 20, Baush & Lomb, Rochester, NY) set at 625 nm for glucose and 540 nm for starch (Renaud and Mauffette, 1991; Haissig and Dickson, 1979).

Woody samples from buttress roots were collected to estimate starch content using Lugol's solution. The Lugol's solution was prepared using 15 g of KI and 3 g of I_2 in 1000 mL of distilled water (Wargo, 1975). The woody sample was cut in half

longitudinally and stained with the Lugol's solution. After 5 minutes, the samples were rinsed with distilled water. The starch content was visually evaluated based on the amount stained and expressed as low, medium, and high.

Additional woody samples (0.5 cm long and 0.3 cm wide) from each tree were collected and place in a small vial with 2.5 mL distilled water. After 24 hours, the glucose content of the solution was determined using a portable refractometer (VEE GEE, Kirkland, WA) and a blood glucose meter (Accu-chek® aviva; Roche, Indianapolis, IN). The refractometer is an optical instrument that can determine the fluid concentrations such as glucose content (Brix level) based on the refractive index of the substance. The blood glucose meter is a medical tool designed to determine glucose concentration in human blood. The meter uses a small drop of blood which is placed on a test strip for calculating blood glucose level. The advantage of using this type of tool is that the glucose content can be easily and quickly determined using a small amount of solution.

Chlorophyll fluorescence Fv/Fm was measured using the HandyPEA® (Hansatech Instruments Ltd, King's Lynn, UK). Chlorophyll fluorescence was measured on leaves randomly collected in the lowest third of the canopy of each tree. Ten leaves from the lowest third of the canopy were adapted to darkness for 25 minutes. After the darkness period, measurements were recorded up to 1 s (Percival and Fraser, 2001; Percival and Fraser, 2005).

Electrical resistances were measured with a field ohmmeter (Shigometer® OZ-67; Osmose Wood Preservatives, Inc. Buffalo, NY) on the trunk at 1 m above ground.

Stainless-steel probes (5 cm long) spaced 1.5 cm apart were inserted vertically through the bark into the cambial zone. Three resistance readings were recorded on the trunk of each tree. Electrical resistances were taken in the early morning to avoid temperature variability (McCullough and Wagner, 1987). All variables were measured during the dormant season (February 2007) and at the beginning of the growing season (April 2007).

Assessment of techniques in different tree groups

An additional group of large live oaks (40 to 50 cm dbh) on the campus at Texas A&M University (30º37'11.76''N, 96º20'3.53''W) were selected to compare the results on trees with varying levels of visual vitality. Fifteen trees were visually classified in three category conditions good, fair, or poor (5 trees per group) (Fig. 6.1). Visual criteria for selecting the trees were based on canopy structure, visible damage, leaf size and color, canopy density, and twig dieback. The same field techniques used on healthy trees in the previous trial were utilized following the same procedures already indicated, and data was collected during April 2007.

Data analysis

Pearson correlations among all variables from laboratory analysis and field methods were developed for each measurement period. Spearman rank correlation coefficients were determined for relationships among the starch content ratings (Lugol's solution) and the other variables. Linear regressions were used in those variables showing a strong relationship. In the case of the second trial, comparisons among groups

were done with nonparametric analysis using Kruskall Wallis test and Wilcoxon rank sum test. Statistical analyses were done using the SPSS software v.13.
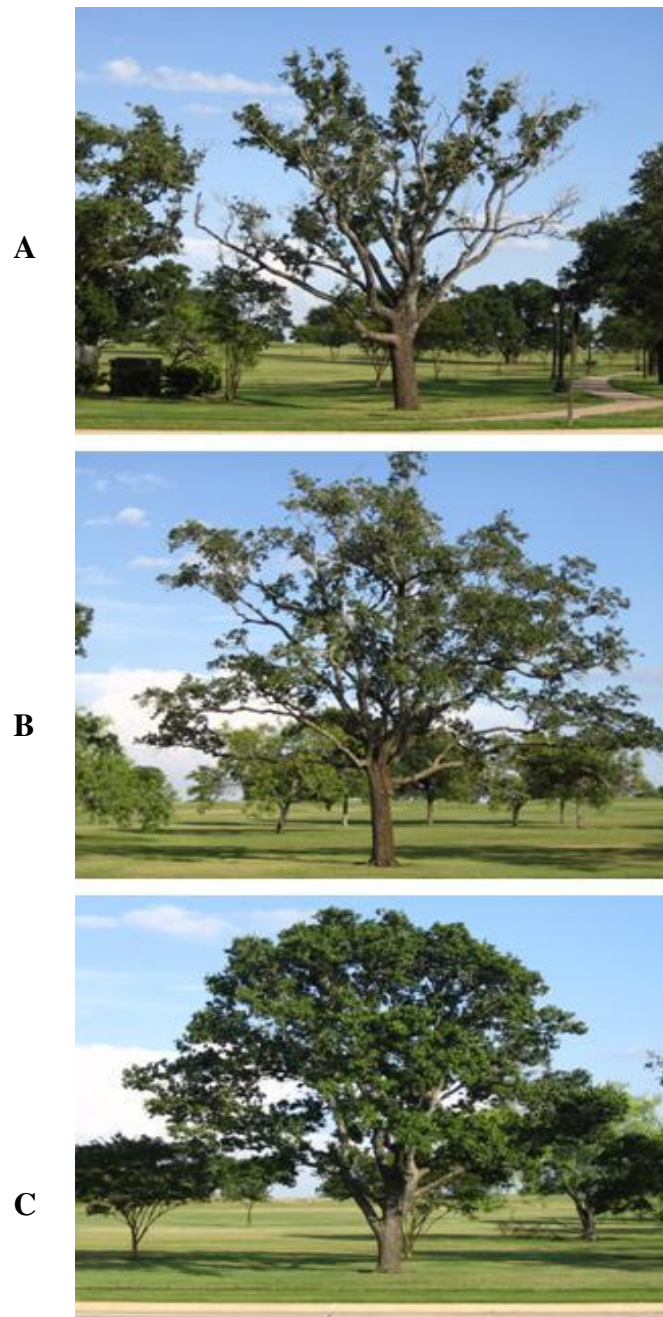

Fig. 6.1. Live oaks near a sidewalk showing different vitality conditions, poor (A), fair (B), and good (C).

Results and Discussion

Comparison of field and laboratory methods

Glucose content can give information which helps to assess tree vitality. Concentrations of glucose can fluctuate widely in response to changing demands by sinks or photosynthesis supply (Schier and Johnston, 1971). In addition, the changes in glucose content in tree tissues can be an indicator of sugar movement associated with phenological stages (Kim and Wetzstein, 2005). There was a strong association during both assessment dates between glucose content determined in the laboratory and the field results using a portable blood glucose meter (Figure 6.2). A portable blood glucose meter appears to offer an easy, accurate, and reliable technique for assessing glucose content in trees and assisting in the quantification of tree vitality.

Table 6.1. Correlation of glucose and starch content from laboratory analysis with Lugol's solution, glucose meter, refractometer, Shigometer®, and chlorophyll fluorescence on two different dates (February and April 2007).

Technique	February		April	
	Glucose	**Starch**	**Glucose**	**Starch**
Lugol's solution	0.011	0.327	0.060	0.442*
Blood Glucose meter	0.766**	-0.116	0.781**	-0.410*
Refractometer	0.103	-0.385*	0.130	-0.074
Shigometer®	-2.246	-0.059	-0.136	-0.008
Chlorophyll fluorescence	0.159	-0.617*	0.168	-0.441*

*Significant correlation at $P<0.05$; ** highly significant correlation at $P<0.01$

The results using the Lugol's stain solution did not show a significant correlation with the starch content from the laboratory during the dormant season (Table 6.1). Even though some trees visually exhibited varying stress symptoms, most samples displayed a

dark purple stain during dormant season. The trees may have had sufficient levels of stored starch so that the technique was not sensitive enough to identify differences among trees. Wargo (1979) found that unstressed sugar maples had high starch contents in their roots during the dormant season. This condition may affect the use of this technique in trees under favorable conditions or during the dormant season. Starch results using Lugol's solution during the growing season was positively correlated with the starch content from the laboratory. Due to the trees' use of stored carbohydrates (starch) at the beginning of the growing season, the differences among trees were more evident in April 2007. However, the results using the Lugol's solution were only useful when comparing trees with wide variations in starch content. McCullough and Wagner (1987) also suggested being careful when comparing results of stained tissues with samples in different season. Previous research using Lugol's solution has found the technique to be a more sensitive indicator of physiological condition in *Acer saccharum* Marsh. when using tiny samples (Carroll et al., 1983).
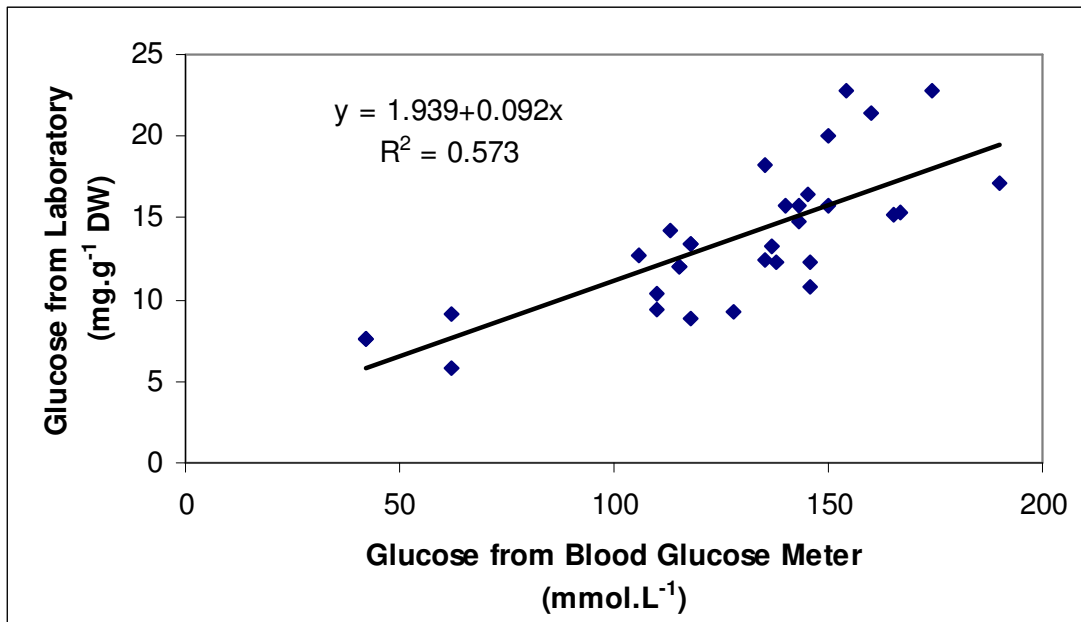
Fig. 6.2. Linear regression between glucose determined with the blood glucose meter and the glucose from laboratory from woody tissues of live oaks.

Glucose from the laboratory did not show significant correlations with results from the refractometer, but starch results demonstrated a negative relationship with refractometer values (Table 6.1). The results may seem counterintuitive considering that the main purpose of using the refractometer was to determine glucose or simple sugars. However, when glucose and starch values from the laboratory analysis were correlated, the results showed a significant negative relationship of -0.464 ($P<0.01$). Linear regression showed significant negative correlations between the variables. Regression data from both assessment dates were significant ($P<0.01$), although data from the dormant season presented a lower R-squared (0.21) than data from the growing season (Fig. 6.3). The results might suggest the possibility of inferring starch content of woody tissues from glucose content. A similar pattern was found in branch tissues of *Pinus*

sylvestris when they compared soluble and insoluble carbohydrates (Kaipiainen and Sofronova, 2003). In addition, an increase in simple sugars and a decrease in starch were detected under stress conditions in *Q. velutina* (Parker and Patton, 1975).
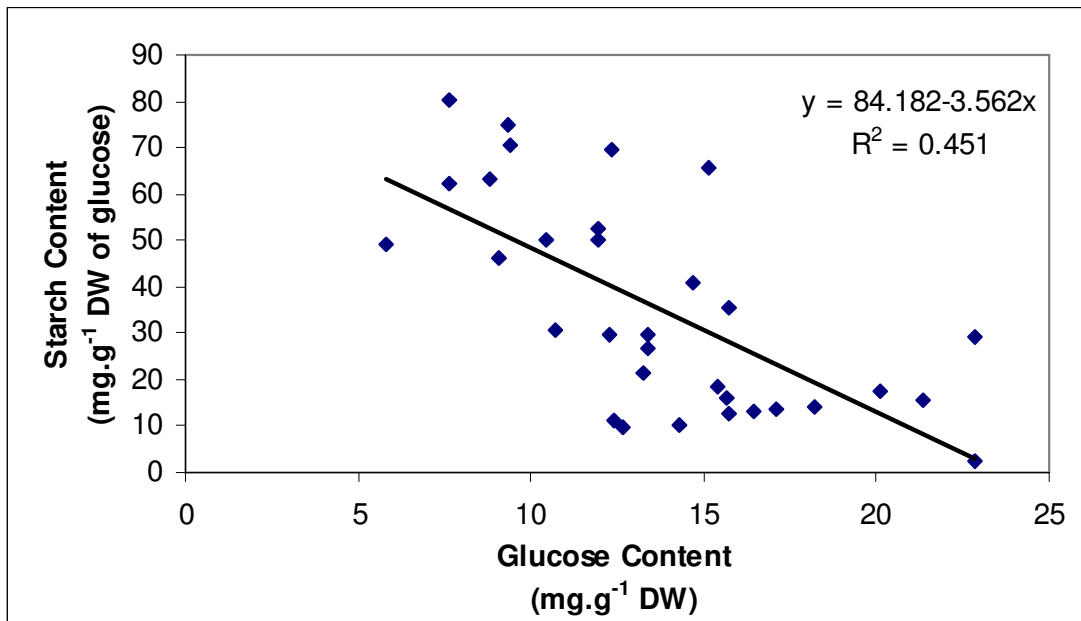
Fig. 6.3. Linear regression among starch and glucose content from the laboratory from samples collected in April from live oaks.

The relationship between starch and glucose content can be caused by the continuous conversion among carbohydrates influenced by environmental or phenological conditions (Pallardy, 2008). When starch stored in roots is converted and translocated to other demanding organs, the tissue may indicate low starch content and high glucose content, while the opposite might occur when translocation is low revealing high starch content and low glucose levels (Kaipiainen and Sofronova, 2003; McLaughlin et al., 1980).

In the case of the Shigometer®, the values did not correlate with any of the other variables used. The use of this technique seems to not be sensitive enough to detect minor vitality changes among trees and may be more suitable for assessing wood decay than physiological functions. Similar results were found in previous studies where the use of the Shigometer® failed to accurately assess vitality in *P. ponderosa* and *Liquidambar styraciflua* L. (Clark et al., 1992; McCulloguh and Wagner, 1987). However, other research indicates that the use of this technique is useful for assessing seasonal variation in species such as *Acer rubrum* L., *Quercus rubra* L., and *Pinus strobus* L. (Davis et al., 1979) or detecting loss of vitality in *Pinus caribaea* Morelet and *P. ponderosa* after stress conditions (McCullough and Wagner, 1987; Paysen et al., 2006). Ostrofsky and Shortle (1989) recommended the use of the Shigometer® with other forestry tools or observations which can provide additional information.

Chlorophyll fluorescence Fv/Fm values during both assessment dates were negatively correlated with starch content (Table 6.1). This result seems contradictory because high chlorophyll fluorescence values indicate a better capacity for leaves to produce carbohydrates and a high potential for translocation to storage tissues (Maxwell and Johnson, 2000). The results may have been affected by cold temperature stress on the leaves during the dormant season. Although results from this trial did not correlate well with other vitality variables, previous research has indicated that chlorophyll fluorescence can be use as a predictor of plant vitality in deciduous trees (Percival, 2004).

Assessment of techniques in different tree groups

Measurements of live oaks with differing vitality conditions varied among different techniques (Table 6.2). Glucose content and Lugol's solution did not show significant differences among different groups. Even though the Lugol's solution did not show significant differences among groups of trees, some of the stained tissue in trees with low starch content were lighter than samples from other groups. As demonstrated in the previous trial, this technique was not sensitive enough to detect small differences in tree vitality.

Table 6.2. Mean values of glucose and starch content from the laboratory, using the blood glucose meter, Lugol's solution, Shigometer®, and chlorophyll fluorescence Fv/Fm in three groups of live oak with varying vitality (poor, fair, and good).

Vitality	Glucose content (mg·g^{-1} DW)	Starch content (mg·g^{-1} of glucose DW)	Blood Glucose meter (mmol·L^{-1})	Lugol's solution	Electrical resistant (KΩ)	Chlorophyll fluorescence Fv/Fm
Poor	16.8a[†]	37.5a	136.2a	2.4a	4.2a	0.82a
Fair	15.1a	37.7a	134.0a	2.0a	3.5a	0.80b
Good	17.9a	13.0b	144.4a	1.6a	2.7b	0.80b

[†]Different letters within columns indicate differences between groups using Wilcoxon rank sum test ($P<0.05$)

In the case of starch content and chlorophyll fluorescence Fv/Fm, the values revealed significant differences between trees exhibiting poor and good health. However, the results were contradictory to the expected results because trees with visually poor vitality had higher starch contents and higher Fv/Fm values. Although this appears counterintuitive, the results may be explained by considering the greater amount of leaves and branches that trees with good vitality had to support. Trees with bigger

canopies might use more carbohydrate reserves in order to maintain a greater number of leaves and branches, which may lower the starch content; however, these trees may compensate with a higher production of carbohydrates from the larger numbers of mature leaves. Visually declining trees may have used fewer reserves in order to maintain the canopy or the carbohydrates were stored as a defense against stressful conditions.

In this study, results from the Shigometer® showed significant differences when comparing good and poor trees. It appears that electrical resistance readings can detect differences in trees that were affected by severe stress conditions (McCullough and Wagner, 1987; Paysen et al., 2006), but it is not sensitive enough for trees with less obvious differences (Clark et al., 1992). Previous research has indicated that Shigometer® readings appear to be most useful during periods of active growth and metabolism (McCullough and Wagner, 1987). Considering that trees exhibiting poor conditions were affected several years ago by construction, results using other techniques might indicate the acclimation process to stressful conditions.

The results from this research indicate that glucose content can be accurately estimated using a portable blood glucose meter. Considering that glucose content was related with starch content, glucose content could be used to broadly assess carbohydrate content in urban trees. This information might be used to compare tree vitality among individuals or group of trees. Unfortunately, carbohydrate content or variables suggested for assessing glucose or starch content did not correlate well with other tools suggested to evaluate vitality in the field. Results indicate that vitality is a complex variable to be

assessed and that visual symptoms are still required in the determination of tree health. Although field techniques used in this study have been suggested for assessing tree vitality, it is important to consider that more than one factor may play a role in the determination of tree vitality (McCullogugh and Wagner, 1987).

CHAPTER VII

SUMMARY

Trees growing in urban environments usually cope with several stressful conditions that negatively impact energy reserves. These stressors, such as pollution, soil contamination, soil compaction, heat islands, and diseases, may reduce carbohydrate production (Pallardy, 2008). The translocation of carbohydrates within a tree is ruled by source-sink relationships that are affected by environmental conditions or developmental stages (Allen et al., 2005). Mature leaves are the main sources of carbohydrates, and roots store high concentrations of sugars, mainly as starch (Taiz and Zeiger, 2006). A lack of carbohydrate production and depletion of stored carbohydrates can cause tree decline or death (Kosola et al., 2001; Wargo et al., 1972). Understanding carbohydrate partitioning in trees can be useful in determining the best place to monitor tree carbohydrate content.

The importance of various carbohydrates on tree vitality has been studied (Abod and Webser, 1991; Gregory and Wargo, 1985; Tainter and Lewis, 1982). Most of these researchers have focused on starch, glucose, and sucrose levels, the primary carbohydrates stored and translocated in trees (Alaoui-Sosse et al., 1994). Although most of these studies pointed out the importance of carbohydrates in tree health, there is little research about the exogenous application of carbohydrates to trees to improve tree health. Understanding the effects of carbohydrate applications such as glucose and

starch, to trees and soil may provide valuable, practical data to assist arborists in rehabilitating declining trees.

Two common methods of applying systemic products to trees are root drenches and trunk injections. Most of the research concerning uptake of soil-applied carbohydrates conducted on crop plants has revealed that root cells are able to uptake carbohydrates from the soil (Stanzel et al., 1988; Stubbs et al., 2004). When applying simple sugars or starch to the root system, the role of soil microbiota should be considered because microorganisms can quickly use the carbohydrates (Jonasson et al., 1996). Research on adding sucrose to the root zones of seedling trees has improved root growth (Percival and Fraser, 2005). As with soil-applied carbohydrates, there is very little research on trunk-injected carbohydrates. Most of these studies have been conducted in crops and fruit trees and have shown little or no effect (Iglesias et al., 2003; Zhou et al., 1997). However, the results might be affected by species, age, or health of the plant or by the formulations and concentrations of carbohydrates applied in prior studies.

Environmental factors may have a negative or positive effect on tree vitality and defining and measuring tree vitality has been a difficult task for researchers. Tree vitality has been described as tree condition or tree health. In most of these cases, tree vitality has been evaluated in terms of growth (Dobbertin, 2005). Some studies have described vitality by measuring carbohydrate levels, mainly the starch content on roots (Wargo, 1976; 1975). Other research recommends the use of field instruments such as chlorophyll fluorescence spectrometers and ohmmeters to assess vitality (Percival, 2004;

Wargo et al., 2002). Practicing arborists typically use leaf color and size, twig growth, and canopy density to approximate tree health. However, the effectiveness of using these techniques in urban trees still needs to be evaluated.

An initial study conducted to study carbohydrate partitioning within live oak (*Quercus virginiana*) revealed that carbohydrate concentrations varied among root, trunk, twig, and leaf tissues sampled within the tree. Annual mean glucose concentration in leaves was 49.55 mg·g^{-1} dry tissue. Glucose concentrations in leaves were almost double those present in twigs, trunks, or roots. Concerning total starch concentrations, roots and trunks tissues annually averaged 38.98 and 38.22 mg·g^{-1} of glucose dry tissue, respectively. These concentrations were approximately three times the starch concentrations found in other tissues. Glucose levels were significantly higher in leaves during the winter, while starch concentrations were significantly higher in root and trunk tissues during the spring and winter assessments.

Field and laboratory studies conducted to study the effects of carbohydrate applications to the soil revealed that the effect of exogenous carbohydrates was not detected through the recovery and enumeration of microbial populations from samples under field or laboratory conditions. Soil respiration was significantly increased by glucose applications under field and laboratory conditions. Results of starch applications under laboratory conditions showed that soil respiration was not significantly affected until the fifth week after treatment application. The starch-induced increase in soil respiration was more evident from samples under field conditions. The increase in soil

respiration from exogenously applied glucose lasted about two to three weeks, while the starch effect lasted for about eight to nine weeks.

Another study to investigate the effects of soil-applied carbohydrates on live oaks revealed that growth and net carbon assimilation were not significantly affected by soil drench applications with glucose, starch or a 50:50 mixture (glucose:starch) at 40, 80, or 120 $g \cdot L^{-1}$. Average chlorophyll fluorescence Fv/Fm values varied among carbohydrates and concentrations throughout the experiment and did not demonstrate a clear trend. Higher carbohydrate applications did influence the concentration of glucose in twigs although the results did not clearly indicate that this effect was caused by an uptake of glucose from the root system or by an indirect effect as a result of an effect on microbial activity. Carbon isotope ratio ($\delta^{13}C$) did not provide any evidence about potential carbohydrate uptake. Even though there was a significant increase in soil respiration after being treated with starch, no significant increase in growth or vitality was detected. In addition, nutrient analysis of soil and leaves did not show differences between the control and the treated samples one year after treatment.

A separate study focused on evaluating the effects of trunk injections with glucose and sucrose on growth and vitality of live oaks. Trunk growth indices revealed a significant difference ($P<0.05$) among the overall mean of carbohydrates, but not for concentrations, which might suggest that either the concentrations were insufficient to affect tree growth or that sugars were used for processes other than growth. The results also indicated that the 50:50 mixture of glucose and sucrose caused a higher effect on overall growth indices means. Root growth and photosynthesis were not significantly

different (P>0.05) among sugars or concentrations. Starch content in twigs and glucose content in roots revealed no significant differences, but glucose content in twigs and starch in roots were significantly different (P<0.05) among overall means for concentrations, demonstrating an increase in trees treated with the highest carbohydrate concentrations (120 g·L^{-1}). Results with chlorophyll fluorescence Fv/Fm showed significant differences (P<0.001) among overall concentrations. The results might indicate that carbohydrate content may be more closely associated with tree vitality than growth since the results among those variables showed a similar trend. The carbon isotopic ratios (δ^{13}C) did not demonstrate significant differences among root samples, but were significantly different (P<0.05) in twigs when comparing some treatments against the control. However, the results did not show a definite trend that corroborated the presence of exogenous carbohydrate sources. Results from this experiment show how annual trunk injections of carbohydrates can significantly affect growth and vitality in live oaks.

Research directed to compare laboratory analysis of carbohydrate content with practical field methods found that there was a strong association between glucose content determined in the laboratory and the field results using a portable blood glucose meter. A portable blood glucose meter appears to offer an easy, accurate, and reliable technique for assessing glucose content in trees. The use of Lugol's stain solution did not show a significant correlation with starch content from the laboratory; however, the Lugol's solutions were useful when comparing trees with wide variations in starch content. Glucose from the laboratory did not show significant correlations with results

from the refractometer, but starch results demonstrated a negative relationship with refractometer. Linear regression revealed significant negative correlations between glucose and starch values from the laboratory. The use of the Shigometer® seems to not be sensitive enough to detect minor vitality changes among trees and may be more suitable for assessing wood decay than physiological functions. Chlorophyll fluorescence Fv/Fm values during both assessment dates were negatively correlated with starch content.

Further assessments of field methods to measure vitality of live oaks with differing levels of vitality varied among the different techniques. Glucose content and Lugol's solution did not show significant differences among different groups. In the case of starch content and chlorophyll fluorescence Fv/Fm, the values revealed significant differences between trees exhibiting poor and good health. In this trial, results from the Shigometer® showed significant differences when comparing good and poor trees indicating that electrical resistance readings can detect differences in trees that were affected by severe stress conditions. Glucose and starch content did not correlate well with tools suggested to evaluate vitality in the field. Results indicate that vitality is a complex variable to be assessed and that visual symptoms are still required in the determination of tree health.

LITERATURE CITED

Abdin, O.A., X. Zhou, B.E. Coulman, D. Cloutier, M.A. Faris, and D.L. Smith. 1998. Effect of sucrose supplementation by stem injection on the development of soybean plants. J. Exp. Bot. 49(329):2013-2018.

Abod, S.A. and A.D. Webster. 1991. Carbohydrates and their effects on growth and establishment of *Tilia* and *Betula*: I. Seasonal changes in soluble and insoluble carbohydrates. J. Hort. Sci. 66(2):235-246.

Abramoff, M.D., P.J. Magelhaes, and S.J. Ram. 2004. Image processing with ImageJ. Biophoton. Intl. 11(7):36-42.

Alaoui-Sosse, B., C. Parmentier, P. Dizengremel, and P. Barnola. 1994. Rhythmic growth and carbon allocation in *Quercus rubur*. 1. Starch and sucrose. Plant Physiol. Biochem. 32(3):331-339.

Alexander, D.B. 2005. Bacteria and Archae. p. 44-71. In: D.M. Sylvia, J.J. Fuhrmann, P.G. Hartel, and D.A. Zuberer (eds.). Principles and applications of soil microbiology. Pearson Prentice Hall, Englewood Cliffs, NJ.

Allen, M.T., P. Prusinkiewicz, and T.M. Dejong. 2005. Using L-systems for modeling source-sink interactions, architecture and physiology of growing trees: the L-PEACH model. New Phytol. 166:869-880.

Anderson, J.P.E. 1982. Soil respiration. p. 831-871. In: A.L. Page, R.H. Miller, and D.R. Keeney (eds.). Methods of soil analysis, Part 2. Chemical and Microbiological Properties. ASA and SSSA, Madison, WI.

Appel, D.N. 2001. The use of Alamo for oak wilt management. p. 101-106. In: C.L. Ash (ed.). Shade tree wilt diseases. APS Press, St. Paul, MN.

Arnold, M.A., G.V. McDonald, D.L. Bryan, G.C. Denny, W.T. Watson, and L. Lombardini. 2007. Below-grade planting adversely affects survival and growth of tree species from five different families. Arboric. Urban For. 33(1):64-69.

Bååth, E. and K. Arnebrant. 1994. Growth rate and response of bacterial communities to pH in limed and ash treated forest soils. Soil Biol. Biochem. 26(8):995-1001.

Barbaroux C., N. Breda, and E. Dufrene. 2003. Distribution of above-ground and below-ground carbohydrate reserves in adult trees of two contrasting broad-leaved species (*Quercus petrea* and *Fagus sylvatica*). New Phytol. 157:605-615.

Blanchard, R.O., W.C. Shortle, and W. Davis. 1983. Mechanism relating cambial electrical resistance to periodic growth rate of balsam fir. Can. J. For. Res. 13:472-480.

Bloem, J. P. Ruiter, and L. Bouwman. 1997. Soil food webs and nutrient cycling in agroecosystems. p. 245-278. In: J.D. van Elsas, J.T. Trevors, and E.M.H. Wellington (eds.). Modern soil microbiology. Marcel Dekker Inc., New York.

Carroll, J.E., T.A. Tattar, and P.M. Wargo. 1983. Relationship of root starch to decline of sugar maple. Plant Dis. 67:1347-1349.

Cheng, X. and C.S. Bledsoe. 2002. Contrasting seasonal patterns of fine root production for blue oaks (*Quercus douglasii*) and annual grasses in California oak woodland. Plant Soil 240:263-274.

Cheshire, M.V. 1979. Nature and origin of carbohydrates in soils. Academic Press, New York.

Chidthaisong, A. and R. Conrad. 2000. Turnover of glucose and acetate coupled to reduction of nitrate, ferric iron and sulfate and to methanogenesis in anoxic rice field soil. FEMS Microbiol. Ecol. 31:73-86.

Clark, J., R. Kjelgran, J. Hushagen, and J. Fiore. 1992. Cambial electrical resistance does not assess vitality of individual sweet gum tress. J. Arboric. 18(1):1-5.

Costonis, A.C. 1981. Tree injections: perspective macro-injection/micro-injection. J. Arboric. 7(10):275-277.

Dassonville, F., J.J. Godon, P. Renault, A. Richaume, and P. Cambier. 2004. Microbial dynamics in an anaerobic soil slurry amended with glucose and their dependence on geochemical processes. Soil Biol. Biochem. 36:1417-1430.

Davis, W., A. Shigo, and R. Weyrick. 1979. Seasonal changes in electrical resistance of inner bark in red oak, red maple, and Eastern white pine. For. Sci. 25(2):282-286.

Dean, T.J. 2001. Potential effect of stand structure on belowground allocation. For. Sci. 47(1):69-76.

DeLucia, E.H., T.W. Sipe, J. Herrick, and H. Maherali. 1998. Sapling biomass allocation and growth in the understory of a deciduous hardwood forest. Amer. J. Bot. 85(7):955-963.

Dobbertin, M. 2005. Tree growth as indicator of tree vitality and of tree reaction to environmental stress: a review. Eur. J. For. Res. 124:319-333.

Domisch, T., L. Finer, and T. Lehto. 2002. Growth, carbohydrate and nutrient allocation of Scots pine seedlings after exposure to simulated low soil temperature in spring. Plant Soil 246:75-86.

Dunn, B.A. and J.A. Rowland. 1986. Needle electrode driver for tree vigor determinations. J. Arboric. 12(4):108-111.

Eggers, J., J. Juzwik, S. Bernick, and L. Mordaunt. 2005. Evaluation of propiconazole operational treatments of oaks for oak wilt control. U.S. Dept. Agric. For. Serv. Resp. North Central Research Station. NC-390.

Eleki, K., R.M. Cruse, and K.A. Albrecht. 2005. Root segregation of C_3 and C_4 species using carbon isotope composition. Crop Sci. 45:879-882.

Farquhar, G.D., J.R. Ehleringer, and K.T. Hubick. 1989. Carbon isotope discrimination and photosynthesis. Annu. Rev. Plant Physiol. Plant. Mol. Biol. 40:503-537.

Filip, G.M., L.M. Ganio, P.T. Oester, R.R. Mason, and B.E. Wickman. 2002. Ten-year effect of fertilization on tree growth and mortality associated with Armillaria root disease, fir engravers, dwarf mistletoe, and Western spruce budworm in North-eastern Oregon. Western J. Appl. For. 17:122-128.

Fotelli, M.N., H. Rennenberg, T. Holst, H. Mayer, and A. Gebler. 2003. Carbon isotope composition of various tissues of beech (*Fagus sylvatica*) regeneration is indicative of recent environment conditions within the forest understory. New Phytol. 159:229-244.

Gansert, D. and W. Sprick. 1998. Storage and mobilization of nonstructural carbohydrates and biomass development of beech seedlings (*Fagus sylvatica* L.) under different light regimes. Trees 12:247-257.

Garcia, G.R., A. Gomez, J. Lopez-U, J. Vargas-H., and W.R. Horwath. 2004. Tree growth and $\delta^{13}C$ among populations of *Pinus greggii* Engelm. at two contrasting sites in central Mexico. Forest Ecol. Manag. 198:237-247.

Giedraitis, J. 1990. Treating the treaty oak. p. 159-163. In: P.D. Rodbell (ed.) Make our cities safe for trees: proceedings of the fourth urban forestry conference. Amer. For. Assn, St. Louis, MO.

Gilman, E.F. and D.G. Watson. 1994. *Quercus virginiana*: southern live oak. U.S. Dept. Agric. For. Serv. Fact Sheet. ST-564. 4 p.

Gregory, R.A. and P.M. Wargo. 1985. Timing of defoliation and its effect on bud development, starch reserves, and sap sugar concentration in sugar maple. Can. J. For. Res. 16:10-17.

Grulke, N.E., C.P. Andersen, and W.E. Hogsett. 2001. Seasonal changes in above-and belowground carbohydrate concentrations of ponderosa pine along a pollution gradient. Tree Physiol. 21:173-181.

Guggenberger G., E.T. Elliott, S.D. Frey, J. Six, and K. Paustian. 1999. Microbial contributions to the aggregation of a cultivated grassland soil amended with starch. Soil Biol. Biochem. 31:407-419.

Gupta, M.C. 1986. Population dynamics of *Fusarium* species in soil amended with carbonaceous and nitrogenous materials. Indian Phytopatol. 39(2):253-258.

Haissig, B.E. and R.E. Dickson. 1979. Starch measurement in plant tissue using enzymatic hydrolysis. Physiol. Plant. 47:151-157.

Harmer, R. and I. Alexander. 1986. The effect of starch amendment on nitrogen mineralization from the forest floor beneath a range of conifers. Forestry 59(1):39-46.

Harris, R.W., J.R. Clark, and N.P. Matheny. 2004. Arboriculture: integrated management of landscape trees, shrubs, and vines. Prentice Hall, Upper Saddle River, NJ.

Hu, S. and A.H.C. van Bruggen. 1997. Microbial dynamics associated with multiphasic decomposition of ^{14}C-labelled cellulose in soil. Microb. Ecol. 33:134-143.

Iglesias, D.J., F.R. Tadeo, E. Primo-Millo, and M. Talon. 2003. Fruit set dependence on carbohydrate availability in citrus trees. Tree Physiol. 23:199-204.

Iglesias, D.J., F.R. Tadeo, F. Legaz, E. Primo-Millo, and M. Talon. 2001. In vivo sucrose stimulation of colour change in citrus fruit epicarps: interactions between nutritional and hormonal signals. Physiol. Plant. 112:244-250.

Illeris, L., and S. Jonasson. 1999. Soil and plant CO_2 emission in response to variations in soil moisture and temperature and to amendment with nitrogen, phosphorus, and carbon in Northern Scandinavia. Arct. Antarct. Alp. Res. 31(3):264-271.

Ilstedt, U., A. Nordgren, and A. Malmer. 2000. Optimum soil water for soil respiration before and after amendment with glucose in humid tropical acrisols and a boreal mor layer. Soil Biol. Biochem. 32:1591-1599.

Jaenicke, H. and M. Thiong'o. 1999. Comparison of two methods for the routine extraction and quantification of non-structural carbohydrates in tree tissues. J. Agr. Sci. Tech. 2(1):46-55.

Jessup, K.E., P.W. Barnes, and T.W. Boutton. 2003. Vegetation dynamics in a *Quercus-Juniperus* savanna an isotopic assessment. J. Veg. Sci. 14:841-852.

Jonasson, S., P. Vestergaard, M. Jensen, and A. Michelsen. 1996. Effects of carbohydrate amendments on nutrient partitioning, plant and microbial performance of a grassland-shrub ecosystem. Oikos 75:220-226.

Kaelke, C.M. and J.O. Dawson. 2005. The accretion of nonstructural carbohydrates changes seasonally in *Alnus incana* ssp. *rugosa* in accord with tissue type, growth, N allocation, and root hypoxia. Symbiosis 39:61-66.

Kaipiainen, L.K. and G.I. Sofronova. 2003. The role of the transport system in the control of the source-sink relations in *Pinus sylvestris*. Russian J. Plant Physiol. 50(1):125-132.

Kent, D., D. Halcrow, T. Wyatt, and S. Shultz. 2004. Detecting stress in southern live oak (*Q. virginiana* var. *geminate*). J. Arboric. 30(3):146-153.

Kim, T. and H.Y. Wetzstein. 2005. Seasonal fluctuations in nutrients and carbohydrates in pecan leaves and stems. J. Hort. Sci. Biotech. 80(6):681-688.

Koch, K. 1996. Carbohydrate modulated gene expression in plants. Annu. Rev. Plant Physiol. Mol. Biol. 47:509-540.

Kolb, T.E. and L.H. McCormick. 1991. Relationship between total nonstructural carbohydrate concentration and root diameter in sugar maple. For. Sci. 37(1):343-346.

Kolosa, K.R., D.I. Dickmann, E.A. Paul, and D. Parry. 2001. Repeated insect defoliation effects on growth, nitrogen acquisition, carbohydrates, and root demography of poplars. Oecologia 129:65-74.

Larcher, W. 1980. Physiological plant ecology. 2nd ed. Springer-Verlag, New York.

Lentner, M. and T. Bishop. 1986. Experimental design and analysis. Valley Book Co., Blacksburg, VA.

Levitt, J. 1980. Responses of plants to environmental stress, Volume 1. Academic Press, New York.

Lilly, S.J. 2001. Arborists' certification study guide. International Society of Arboriculture, Champaign, IL.

Little, E.L. 1979. Checklist of United States trees (native and naturalized). U.S. Dept. Agric. For. Serv. Handbook 541. Washington, D.C.

Ludovici, K.H., H.L. Allen, T.J. Albaugh, and P.M. Dougherty. 2002. The influence of nutrient and water availability on carbohydrate storage in loblolly pine. Forest Ecol. Manage. 159:261-270.

Maxwell, K. and G.N. Johnson. 2000. Chlorophyll fluorescence - a practical guide. J. Exp. Bot. 51(354):659-668.

Mayhead, G.J. 1991. Observations on the use of high pressure tree injection system. Arboric. J. 15:19-26.

McCullough, D.G. and M.R. Wagner. 1987. Evaluation of four techniques to assess vigor of water-stressed ponderosa pine. Can. J. For. Res. 17:138-145.

McLaughlin, S.B., R.K. McConathy, R. L. Barnes, and W.T. Edwards. 1980. Seasonal changes in energy allocation by white oak (*Quercus alba*). Can. J. For. Res. 10:379-388.

Michelsen, A., E. Graglia, I.K. Schmidt, S. Jonasson, D. Sleep, and C. Quarmby. 1999. Differential responses of grass and dwarf shrub to long-term changes in soil microbial C, N and P following factorial addition of NPK fertilizer, fungicide and labile carbon to a heath. New. Phytol. 143:523-538.

Montes, N., T. Gauquelin, W. Badri, V. Bertaudiere, and E.H. Zaoui. 2000. A non-destructive method for estimating above-ground forest biomass in threatened woodlands. Forest Ecol. Manage. 130:37-46.

Narog, M.G., T.E. Paysen, B.M. Corcoran, and M.A. Zavala. 1997. Monitoring fire injury in Canyon live oak with electrical resistance. p. 646-647. In: N.H. Phillsbury, J. Verner, and D. William (eds.). Proceedings of a symposium on oak woodlands: ecology, management and urban interface issues. USDA For. Serv. Pac. Southwest Res. Sta. Gen. Tech. Rep. PSW-GTR-160, Berkeley, CA.

Nelson, D.L. and M.M. Cox. 2005. Lehninger principles of biochemistry. W.H. Freeman and Co., New York.

Newell, E.A., S.S. Mulkey, S.J. Wright. 2002. Seasonal patterns of carbohydrates storage in four tropical tree species. Oecologia. 131:333-342.

Nguyen, P.V., D.I. Dickmann, K.S. Pregitzer, and R. Hendrick. 1990. Late-season changes in allocation of starch and sugar to shoots, coarse roots, and fine roots in two hybrid poplar clones. Tree Physiol. 7:95-105.

Osterbauer, N.K. and D.W. French. 1992. Propiconazole as a treatment for oak wilt in *Quercus rubra* and *Q. ellipsoidalis*. J. Arboric. 18(5):221-226.

Ostrosfsky W.D. and W.C. Shortle. 1989. Applications of the Shigometer for assessing tree and forest health and wood product quality-a review. Rev. Trop. Plant Path. 6:39-57.

Pallardy, S.G. 2008. Physiology of woody plants. 3rd ed. Academic Press, New York.

Papavizas, G.C., J.A. Lewis, and P.B. Adams. 1968. Survival of root-infecting fungi in soil. II. Influence of amendment and soil carbon-to-nitrogen balance on fusarium root rot of beans. Phytopathol. 58:365-372.

Parker, J. and R.L. Patton. 1975. Effects of drought and defoliation on some metabolites in roots of black oak seedlings. Can. J. For. Res. 5:457-463.

Parkinson, D. 1982. Filamentous fungi. p. 949-968. In: A.L. Page, R.H. Miller, and D.R. Keeney (eds.). Methods of soil analysis, Part 2. Chemical and Microbiological Properties. ASA and SSSA, Madison, WI.

Pascual, J.A., C. Garcia and T. Hernandez. 1999. Lasting microbiological and biochemical effects of the addition of municipal solid waste to an arid soil. Biol. Fertil. Soils 30:1-6.

Pascual, J.A., C. Garcia, T. Hernandez, and M. Ayuso. 1997. Changes in the microbial activity of an arid soil amended with urban organic wastes. Biol. Fert. Soils 24:429-434.

Pate, J.S. and T.E. Dawson. 1999. Assessing the performance of woody plants in uptake and utilization of carbon, water and nutrients. Agrofor. Syst. 45:245-275.

Paysen, T.E., A.L. Koonce, E. Taylor, and M.O. Rodriguez. 2006. Indications of vigor loss after fire in Caribbean pine (*Pinus caribaea*) from electrical resistance measurements. Int. J. Wildland Fire 15:415-425.

Percival, G.C. 2004. Evaluation of physiological test as predictors of young tree establishment and growth. J. Arboric. 30(2):80-91.

Percival, G.C. and S. Boyle. 2005. Evaluation of microcapsule trunk injections for the control of apple scab and powdery mildew. Ann. Appl. Biol. 147:119-127.

Percival, G.C. and G.A. Fraser. 2001. Measurement of the salinity and freezing tolerance of *Crataegus* genotypes using chlorophyll fluorescence. J. Arboric. 27(5):233-245.

Percival, G.C. and G.A. Fraser. 2005. Use of sugars to improve root growth and increase transplant success of birch (*Betula pendula* Roth). J. Arboric. 31(2):66-77.

Percival, G.C., G.A. Fraser, and S. Barnes. 2004. Soil injections of carbohydrates improve fine root growth of established urban trees. Arboric. J. 28:1/2:95-101.

Percival, G.C. and C.N. Sheriffs. 2002. Identification of drought-tolerant woody perennials using chlorophyll fluorescence. J. Arboric. 28(5):215-223.

Percival, G.C. and T. E. Smiley. 2002. One lump or two. Grounds Maint. 37(4):18-20.

Perry, T.O., F.S. Santamour Jr., R.J. Stipes, T. Shear, and A.L. Shigo. 1991. Exploring alternatives to tree injection. J. Arboric. 17(6):217-226.

Peterson, J.P. and B. Fry. 1987. Stable isotopes in ecosystem studies. Ann. Rev. Ecol. Systemat. 18:293-320.

Polak, T., B.N. Rock, P.E. Campbell, J. Soukupova, B. Solcova, K. Zvara, and J. Alberchtova. 2006. Shoot growth processes, assessed by bud development types, reflect Norway spruce vitality and sink prioritization. Forest Ecol. Manag. 225:337-348.

Renaud, J.P. and Y. Mauffette. 1991. The relationship of crown dieback with carbohydrate content and growth of sugar maple (*Acer saccharum*). Can. J. For. Res. 21:1111-1118.

Retzlaff, W.A., J.A. Handest, D.M. O'Malley, S.E. McKeand, and M.A. Topa. 2001. Whole-tree biomass and carbon allocation of juvenile trees of loblolly pine (*Pinus taeda*): influence of genetics and fertilization. Can. J. For. Res. 31:960-970.

Riek J., A. Piqueras, and P.C. Debergh. 1997. Sucrose uptake and metabolism in a double layer system for micropropagation of *Rosa multiflora*. Plant Cell Tiss. Org. 47:269-278.

Ros, M., M.T. Hernandez, and C. Garcia. 2003. Soil microbial activity after restoration of a semiarid soil by organic amendments. Soil Biol. Biochem. 35:463-469.

Sachs R.M., G. Nyland, W.P. Hackett, J. Coffelt, J. DeBie, and G. Gianinni. 1977. Pressurized injection of aqueous solutions into tree trunks. Scientia Hort. 6:297-310.

Samuelson L.J. and J.M. Kelly. 1996. Carbon partitioning and allocation in northern red oak seedling and mature trees in response to ozone. Tree Physiol. 16:853-858.

Sanchez-Zamora, M.A. and R. Fernandez-Escobar. 2000. Injector-size and the time of application affects uptake of tree trunk-injected solutions. Scientia Hort. 84:163-177.

Sanchez-Zamora, M.A. and R. Fernandez-Escobar. 2004. Uptake and distribution of trunk injections in conifers. J. Arboric. 30(2):73-79.

Sanginga, N., K. Mulongoy, and M.J. Swift. 1992. Contribution of soil organisms to the sustainability and productivity cropping systems in the tropics. Agr. Ecosyst. Environ. 41:135-152.

Schier, G.A. and R.S. Johnston. 1971. Clonal variation in total nonstructural carbohydrates of trembling aspen roots in three Utah areas. Can. J. For. Res. 1:252-255.

Schmidt, I.K., A. Michelsen, and S. Jonasson. 1997a. Effects on plant production after addition of labile carbon to arctic/alpine soils. Oecologia 112:305-313.

Schmidt, I.K., A. Michelsen, and S. Jonasson. 1997b. Effects of labile soil carbon on nutrient partitioning between an arctic graminoid and microbes. Oecologia 112:557-565.

Schmidt, I.K., L. Ruess, E. Bååth, A. Michelsen, F. Ekelund, and S. Jonasson. 2000. Long-term manipulation of the microbes and microfauna of two subarctic heaths by addition of fungicide, bactericide, carbon and fertilizer. Soil Biol. Biochem. 32:707-720.

Sestak, Z. and P. Stiffel. 1997. Leaf age-related differences in chlorophyll fluorescence. Photosynthetica 33 (1-4):347-369.

Shigo, A.L. and W.C. Shortle. 1985. Shigometry: a reference guide. USDA For. Serv. Agric. Handbook No. 646.

Sinton, P., T. Seastedt, B. Bennett, C. Bock, and J. Bock. 2000. C_3 and C_4 species changes identified by $\delta^{13}C$ values of soil organic matter in a Colorado prairie. Western North Am. Nat. 60(4):445-450.

Smith, J.L. and E.A. Paul. 1990. The significance of soil microbial biomass estimations. p. 357-396. In: Bollag, J.M. and G. Sotosky (eds.). Soil biochemistry, vol. 6. Marcel Dekker, New York.

Spoehr, H.A. 1942. The culture of albino maize. Plant physiol. 17:397-410.

Srivastava, N.K. and A.K. Srivastava. 2006. Utilization of exogenously ^{14}C-saccharose into primary metabolites and alkaloid production in *Catharanthus roseus*. Photosynthetica 44(3):478-480.

Stanzel, M., R.D. Sjolund, and E. Komor. 1988. Transport of glucose, fructose and sucrose by *Streptanthus tortuosus* suspension cells. I. Uptake at low sugar concentration. Planta 174:201-209.

Stock, W.D., D.K. Chuba, and G.A. Verboom. 2004. Distribution of South African C_3 and C_4 species of Cyperaceae in relation to climate and phylogeny. Austral Ecol. 29:313-319.

Stubbs, V.E.C., D. Standing, O.G.G. Knox, K. Killham, A.G. Bengough, and B. Griffiths. 2004. Root border cells take up and release glucose-C. Ann. Bot. 93:221-224.

Tainter, F.H. and R. Lewis Jr. 1982. Nonstructural carbohydrate contents of trees affected with Texas live oak decline. Plant Dis. 66:120-122.

Taiz, L. and E. Zeigher. 2006. Plant physiology. 4th ed. Sinauer Associates Inc., Sunderland, MA.

Tarpley, L., D.M. Vietor, and F.R. Miller. 1994. Internodal compartmentalization of stem infused ^{14}C sucrose in sweet and grain sorghum. Crop Sci. 43:1116-1120.

Tate, R. L. 2000. Soil microbiology. 2nd ed. John Wiley & Sons Inc., New York.

Tattar, T.A. and S.J. Tattar. 1999. Evidence for the downward movement of materials injected into trees. J. Arboric. 25(6):325-328.

Taylor, E.L. 1998. Using small-format stereo photogrammetry to characterize changes in the 3D crown architecture of loblolly pine (*Pinus taeda*). Texas A&M Univ., College Station, Ph.D. Diss.

Tognetti, R. and J.D. Johnson. 1999. The effect of elevated atmospheric CO_2 concentration and nutrient supply on gas exchange, carbohydrates and foliar phenolic concentration in live oak (*Quercus virginiana* Mill) seedlings. Ann. For. Sci. 56:379-389.

Tramier, R. and C. Antonini. 1977. Effect of soil amendment with chitin and starch against *Fusarium oxysporum* f. sp. *Dianthi*. Acta Hortic. 71:127-129.

Tromp, J. 1983. Nutrient reserves in roots of fruit trees in particular carbohydrates and nitrogen. Plant Soil 71:401-413.

Tschaplinski, T.J. and T.J. Blake. 1994. Carbohydrate mobilization following shoot defoliation and decapitation in hybrid poplar. Tree Physiol. 14:141-151.

Waes, C.Van, J. Baert, L. Carlier, and E.V. Bockstaele. 1998. A rapid determination of total sugar content and the average inulin change length in roots of chicory (*Cichorium intybus* L.). J. Sci. Food Agr. 76:107-110.

Wagner G.H. and D.C. Wolf 2005. Carbon transformation and soil organic matter formation. p. 219-258. In: D.M. Sylvia, J.J. Fuhrmann, P.G. Hartel, and D.A. Zuberer (eds.). Principles and applications of soil microbiology. Pearson Prentice Hall, Englewood Cliffs, NJ.

Walcroft, A.S., W.B. Silvester, D. Whitehead, and F.M. Kelliher. 1997. Seasonal changes in stable carbon isotope ratios within annual rings of *Pinus radiata* reflect environmental regulation of growth processes. Aust. J. Plant. Physiol. 24:57-68.

Wardle, D.A. and D. Parkinson. 1990. Response of the soil microbial biomass to glucose, and selective inhibitors, across a soil moisture gradient. Soil. Biol. Biochem. 22(6):825-834.

Wargo, P.M. 1975. Estimating starch content in roots of deciduous trees- a visual technique. U.S. Dept. Agric. For. Serv. Res. Pap. NE. 313. Upper Darby, PA.

Wargo, P.M. 1976. Variation of starch content among and within roots of red and white oak trees. For. Sci. 22:468-471.

Wargo, P.M. 1979. Starch storage and radial growth in woody roots of sugar maple. Can. J. For. Res. 9:49-56.

Wargo, P.M., R. Minocha, B.L. Wong, R.P. Long, S.B. Horsley, and T.J. Hall. 2002. Measuring changes in stress and vitality indicators in limed sugar maple on the Allegheny Plateau in north-central Pennsylvania. Can. J. Res. 32:629-641.

Wargo, P.M., J. Parker, and D.R. Houston. 1972. Starch content in roots of defoliated sugar maple. For. Sci. 18:203-204.

Webb, W.L. 1981. Relation of starch content to conifer mortality and growth loss after defoliation by the Douglas-fir tussock moth. For. Sci. 27(2):224-232.

Weston, D., A. Shigo, and R. Weyrick. 1979. Seasonal changes in electrical resistances of inner bark in red oak, red maple, and Eastern white pine. For. Sci. 25(2):282-286.

Worley, R.E. and R.H. Littrell. 1981. Factors affecting injection rate for pressure trunk injection of pecan trees. Scientia Hort. 14:323-328.

Wu, J., P.C. Brookes, and D.S. Jenkinson. 1993. Formation and destruction of microbial biomass during the decomposition of glucose and ryegrass in soil. Soil Biol. Biochem. 25(10):1435-1993.

Zhou, X.M., P. Dutilleul, A.F. MacKenzie, C.A. Madramootoo, and D.L. Smith. 1997. Effects of stem-injected sucrose on grain production, dry matter distribution, and chlorophyll fluorescence of field-grown corn plants. J. Agron. Crop Sci. 178:65-71.

Zhou, X.M. and D.L. Smith. 1996. A new technique for continuous injection into stems of field-grown corn plants. Crop Sci. 36:452-456.

Zuberer, D.A. 1994. Recovery and enumeration of viable bacteria. p. 119-144. In: S.H. Mickelson and J.M. Bigham (eds.). Methods of soil analysis, part 2. Microbiological and biochemical properties, Book no. 5. SSSA, Madison, WI.

VITA

Tomás Martínez Trinidad received a B.S. degree in Forestry at the Universidad Autónoma Chapingo in México in 1996, and in 2000, he received a M.S. degree in Silviculture at the Colegio de Postgraduados in México. While at the Colegio de Postgraduados, his research focused on the effects of water deficits in different populations of *Pinus leiophylla*. After completing an M.S. degree, he worked as an assistant researcher in the Department of Forest Science at the Colegio de Postgraduados.

During his Ph.D. studies, he studied the effect of exogenous applications of carbohydrates on the growth and vitality of urban trees. He also participated in research studying various treatments for leafy mistletoe and the use of rhizotrons to study root diseases. He has presented his M.S. and Ph.D. research at several scientific meetings and has been published in peer-reviewed research journals. Upon completion of his Ph.D. studies, he is planning to continue research on Arboriculture and Urban Forestry issues.

He may be contacted at Colegio de Postgraduados; Campus Montecillo, Programa Forestal; Km. 36.5 Carr. Méx.-Tex. Montecillos; Texcoco, Edo. de México, México. C.P. 56230. Website: www.cem.colpos.mx/cem/. His e-mail is tomtz@colpos.mx.

His permanent mailing address is Calle Vicente Guerrero No. 43; Perote, Veracruz; México. C.P. 91270.