



## Carbohydrate Injections as a Potential Option to Improve Growth and Vitality of Live Oaks

Tomás Martínez-Trinidad, W. Todd Watson, Michael A. Arnold,  
Leonardo Lombardini, and David N. Appel

**Abstract.** This study evaluates the effects of carbohydrate injections on the growth and vitality of live oak (*Quercus virginiana* P. Miller). Glucose, sucrose, or a 50:50 mixture of both carbohydrates at increasing concentrations [0, 40, 80, and 120 g/L (0, 5.3, 10.6, and 16.0 oz/gal)] were injected into live oaks. Trunk and root growth, net photosynthesis, root and twig carbohydrate concentration, and chlorophyll fluorescence were monitored. Isotope composition of twig and root samples was measured as an indicator of injected carbohydrate distribution. There were significant differences ( $P < 0.05$ ) in trunk growth among types of carbohydrates, but no significant differences for carbohydrate concentrations. The mixtures of sucrose and glucose had the largest effect on growth compared to either sugar alone, suggesting that glucose and sucrose alone were used in processes other than trunk growth. 50:50 mixtures caused a greater effect on overall mean growth indices than either sugar alone. Glucose content in twigs and starch in roots were significantly different ( $P < 0.05$ ) among overall means for concentrations with increased levels found in trees treated with the greatest concentrations. Chlorophyll fluorescence Fv/Fm revealed highly significant differences ( $P < 0.001$ ) among overall concentrations. Carbon isotope values did not reveal a definite trend that corroborated the exogenous carbohydrate distribution. Results from this experiment suggest that carbohydrate trunk injections can have an impact on growth and vitality of live oak.

**Key Words.** Glucose; *Quercus virginiana*; Sucrose; Sugars; Tree Vitality.

Photosynthesis in leaves and other chlorophyll-containing tissues produces carbohydrates, which are converted into energy by respiration (Pallardy 2008). Carbohydrates can be used *in situ*, or be 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 (Wargo et al. 1972; Kolosa et al. 2001). 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 (Wargo 1975; Carroll et al. 1983; Percival and Smiley 2002). Use of inexpensive, nontoxic, and environmentally friendly products such as sugars could help improve growth and vitality of trees (Percival 2004; Martínez-Trinidad et al. 2009b).

Trunk injections 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 high amounts of solutions into trees while producing minimal damage (Appel 2001; Eggers et al. 2005). This method makes it easier to control the amount of sugars injected when using greater volumes of solution compared with 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). For sucrose microinjections in fruit trees, research has shown quite variable and unpredictable supplementation of sucrose into the tree by the microinjection system (Iglesias et al. 2001). Anecdotal reports of sucrose macroinjections in the trunk of a large, historic live oak (*Quercus virginiana* P. Miller) 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 tree vitality (Dobbertin 2005). The application of carbohydrates through trunk injections may increase the energy pool and generate greater growth rates (Giedraitis 1990). Injected solutions may move up through the xylem, or they may be stored or translocated to storage 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. The chlorophyll fluorescence parameter Fv/Fm is often used for measuring the photochemical efficiency of photosystem II, which indicates the energy level absorbed by chlorophyll and damage by excess light (Maxwell and Johnson 2000). The Fv/Fm parameter has been suggested as one measurement of tree stress tolerance and tree vitality (Percival and Sheriffs 2002; Percival and Fraser 2005). 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 moved up through the canopy (Tattar and Tattar 1999; Percival and Fraser 2005). The effect may be less evident if 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.

Sugars extracted from  $C_3$  and  $C_4$  plants differ in their carbon isotope ratios  $\delta^{13}C$  (Fotelli et al. 2003) and when sugar from a  $C_4$  plant (e.g., *Zea mays* L.) is applied to a  $C_3$  plant (e.g., *Q. virginiana*), the fate of the applied sugar can potentially be traced within the plant by comparing carbon isotope ratios of treated and nontreated plants. 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 potential 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 [16–20 cm (6.3–7.8 in) dbh] grown under similar conditions were used. Similar trees were selected from a group of nonirrigated trees planted with 6 m (19.6 ft) spacing in an urban forest near College Station, TX in Burleson County (30°33'14.71"N, 96°25'33.61"W). Trees were growing in a Weswood silty clay loam soil. The site has an annual mean temperature of 20.3°C (68.5°F), [-1.6°C (29°F) minimum and 37.7°C (100°F) maximum], and annual precipitation varies between 762 and 1016 mm (30 and 40 in).

Trunk injections using corn-derived glucose, sucrose, or a 50:50 mixture of glucose and sucrose by weight in three different concentrations [40, 80, and 120 g/L (5.3, 10.6, 16.0 oz/gal)] 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 (McLaughlin et al. 1980; Abdin et al. 1998; Iglesias et al. 2003). Approximately 10 L (2.6 gal) 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 (12 in) 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 varying trunk sizes among trees, a growth index was calculated per year by dividing the absolute increase in trunk diameter in a year by the initial trunk measurement at the beginning of the experiment (Arnold et al. 2007). Growth index values were used for the statistical analysis.

Four soil holes [15 cm (6 in) deep x 6 cm (2.3 in) diameter] were dug 1.5 m (4.9 ft) 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

**Table 1. P values from the ANOVA table for diameter growth index, twig glucose content, root starch content, 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/L-1).**

Factors	Diameter growth index	Twig glucose content	Root starch content	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
Concentration x carbohydrate	0.532	0.404	0.152	0.216
Concentration 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

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 (January, April, and August) for carbohydrate analysis. 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 (0.016 fl oz) aliquot of the extract and the glucose standards were mixed with 5 mL (0.16 fl oz) of anthrone reagent (Jaenicke and Thiong'o 1999). Starch content was determined by enzymatic conversion of starch to glucose using amyloglucosidase enzyme in the remaining pellet after glucose extraction. Absorbance of samples and standards were 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; Martinez-Trinidad et al. 2009a).

Net carbon assimilation was measured in each treatment using a portable photosynthesis closed 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.

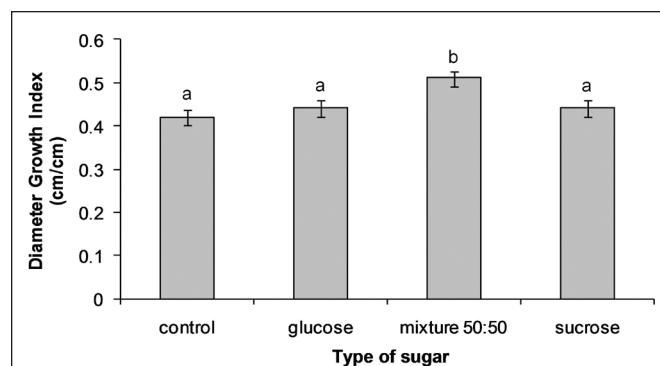
Chlorophyll fluorescence was measured using a HandyPEA® portable fluorescence spectrometer (Hansatech Instruments Ltd, King's Lynn, UK). Ten leaves from the lower two-thirds of the canopy were adapted to darkness for 25 minutes. After the darkness period, the fluorescence response was induced by a red light of 1500  $\mu\text{mol}/\text{m}^2/\text{Hz}$  photosynthetically active radiation intensity provided by an array of 6 light-emitting diodes, with a data acquisition rate of 10  $\mu\text{s}$  for the first 2 ms and 12-bit resolution. The ratio Fv/Fm was used to estimate tree vitality (Percival and Fraser 2001). Chlorophyll fluorescence data was taken at January, April, and August 2005, and January, April, August 2006, and January 2007.

The translocation of carbohydrates was evaluated by determining carbon isotope compositions. Twigs one-year-old and buttress roots samples [4 mm (0.15 in) x 100 mm (3.93 in)] were collected from controls, glucose [40 and 120 g/L (5.3 and 16.0 oz/gal)], and sucrose [40 and 120 g/L (5.3 and 16.0 oz/gal)] treatments 12 months after the first treatment. Samples were submitted for analysis to the Stable Isotope Facility at University of California, Davis. The isotope composition was expressed to Pee Dee Belemnite (PDB) carbonate standard (Peterson and Fry 1987).

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

## RESULTS AND DISCUSSION

Trunk growth revealed a significant difference ( $P < 0.05$ ) among carbohydrates (Figure 1), but not for concentrations. 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, exogenous carbohydrates may have been used for other functions such as storage, defense, or reproduction (Pallardy 2008). Iglesias et al. (2003) found that fruit set in Satsuma mandarin [*Citrus unshiu* (Mak.) Marc., cv. Okitsu] increased by 10% when supplemented with sucrose. Early studies showed that albino corn (*Zea mays* L) survived and produced inflorescences with supplementation of sucrose through the cut ends of leaves (Spoehr 1942).



**Figure 1.** Trunk diameter growth indices (cm/cm) of live oaks injected with three different types of sugars (glucose, sucrose, and a 50:50 mixture). Bars indicate  $\pm 1$  standard error. Different letters between types of sugar indicate significant differences ( $P < 0.05$ ) using LSD.

The results also indicate the 50:50 mixture of glucose and sucrose resulted in a small but significant increase in growth index as compared to sucrose or glucose alone (Figure 1). Sucrose is the main sugar translocated by phloem, while glucose is a simple sugar product of photosynthesis and the base unit of storage carbohydrates (Taiz and Seizer 2006). Trunk injections of more than one type of sugar in live oaks might have an additive 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 (40 oz/gal) (Zhou et al. 1997; Abdin et al. 1998). The amount injected and size of plants might play an important role in the potential effect of carbohydrates injected. In addition, research

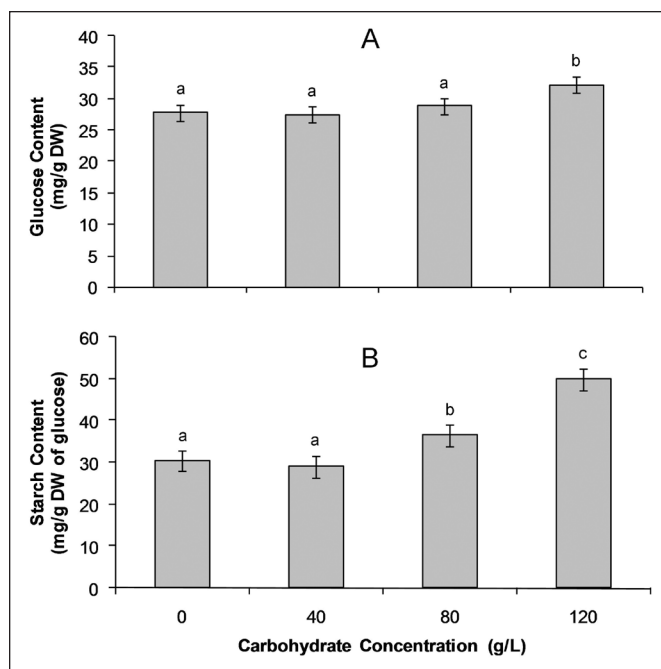
indicates that carbohydrates such as sucrose and glucose can affect sugar sensing systems that initiate changes in gene expression, which can cause an effect on plant growth (Koch 1996).

Results for root growth did not reveal significant differences ( $P > 0.05$ ) among type of sugars or concentrations. It seems the effect of injections was greater 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 due to high variability among samples. Previous research with soybean [*Glycine max* (L.) Merr.] and birch (*Betula pendula* Roth.) 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 and Fraser 2005).

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 affected the analysis. Also, trunk injections were performed during the dormant season with old leaves present before new leaf emergence, which could have reduced the potential effect on photosynthesis. In soybean plants, Abdin et al. (1998) found that the supplementation with sucrose by injections suppressed photosynthesis.

Glucose content in twigs and starch in roots were significantly greater in trees receiving the highest concentration of carbohydrate (Figure 2). This result was not unexpected due to the potential for translocation of exogenously applied carbohydrates upward and/or downward from the injection point (Tattar and Tattar 1999). Prior research showed  $^{14}\text{C}$  sucrose infused into sorghum [*Sorghum bicolor* (L.) Moench] via a pulse application can move upwards through the xylem (Tarpley et al. 1994). Corn plants (*Z. mays*) formed abundant starch when treated with solutions of glucose or sucrose (Spoehr 1942). Similar results were also found in Satsuma mandarin injected with sucrose, which resulted in increased levels of starch in fine roots (Iglesias et al. 2003). The impact of carbohydrate concentrations in this study was more evident in roots where the greatest concentrations [120 g/L (16 oz/gal)] resulted in greater starch levels compared to the control (Figure 2b). Exogenous carbohydrates could have been either stored or translocated to the roots (Tattar and Tattar 1999). High carbohydrate concentrations in other organs like roots and fruits have been reported for Satsuma mandarin when sucrose was injected in the trunk (Iglesias et al. 2003).

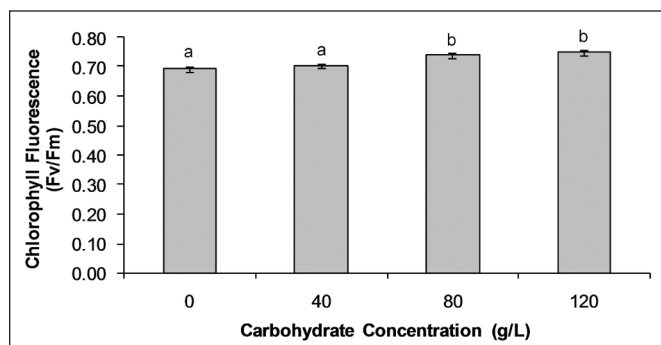
Chlorophyll fluorescence measures the photochemical efficiency of photosystem II (Maxwell and Johnson 2000) and is used as a nondestructive diagnostic for plant vitality and stress (Percival and Sheriffs 2002; Percival 2004; Percival and Boyle 2005). In this study, supplementing trees with carbohydrates via trunk injections increased Fv/Fm (Figure 3), which suggests a method to improve live oak vitality. In addition to chlorophyll fluorescence, similar trends were observed in glucose content in twigs and starch content in roots in response to carbohydrate injection, both used as indicators of tree health (Gregory and Wargo 1985; Wargo et al. 2002; Dobbertin 2005). However, given the increase in trunk diameter by only the sugar mixture treatment, a concomitant response in Fv/Fm and photosynthesis can be expected which may have explained, in part, by the resultant increase in trunk diameter. Growth of many temperate trees is dependent on stored labile carbon produced via photo-



**Figure 2.** Effects of trunk-injected carbohydrates (glucose, sucrose, and a 50:50 mixture) at four concentrations [0, 40, 80, and 120 g/L (0, 5.3, 10.6, and 16 oz/gal)] on glucose content from twigs (A) and starch content from roots (B) of live oaks. Bars indicate  $\pm 1$  standard error. Different letters between types of sugar indicate significant differences ( $P < 0.05$ ) using LSD.

synthesis in previous years (Barford et al. 2001; Gough et al. 2008). Injections of carbohydrate in this study may have been stored for future use instead of utilized immediately for growth.

Carbon isotopic ratios ( $\delta^{13}\text{C}$ ) in roots did not differ ( $P > 0.05$ ) among carbohydrates treatments (data not shown), but there was a significant difference ( $P < 0.05$ ) in the twigs of trees receiving 120 g/L (16 oz/gal) ( $\delta^{13}\text{C} = -29.157\text{‰}$ ) relative to the control ( $\delta^{13}\text{C} = -30.530\text{‰}$ ) suggesting the presence of exogenous carbohydrates in twigs. The lack of difference in  $\delta^{13}\text{C}$  in twigs of trees receiving glucose indicates that glucose was metabolized differently from sucrose.



**Figure 3.** Effects of carbohydrates (glucose, sucrose, and a 50:50 mixture) supplemented by trunk injections at four concentrations [0, 40, 80, and 120 g/L<sup>-1</sup> (0, 5.3, 10.6, and 16 oz/gal)] on chlorophyll fluorescence (Fv/Fm) on live oaks. Bars indicate  $\pm 1$  standard error. Different letters between type of sugars indicate significant differences ( $P < 0.05$ ) using LSD.

Results from this experiment showed how annual trunk injections of carbohydrates during dormancy may improve growth and vitality in live oaks. No visual or physiological damage apart from the injection sites 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 during the growing season should be conducted where the impact on tree performance may be more pronounced.

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*Tomás Martínez-Trinidad (corresponding author)*  
*Colegio de Postgraduados – Programa Forestal*  
*Km. 36.5 Carr. Mex-Tex. Montecillo*  
*Texcoco, Edo. de Mexico. 56230. Mexico.*  
*tomtzt@colpos.mx*

*W. Todd Watson*  
*Department of Ecosystem Science & Management*  
*Texas A&M University*  
*College Station, TX 77843-2138, U.S.*  
*t-watson@tamu.edu*

*Michael A. Arnold*  
*Department of Horticultural Sciences*  
*Texas A&M University*  
*College Station, TX 77843-2133, U.S.*  
*ma-arnold@tamu.edu*

*Leonardo Lombardini*  
*Department of Horticultural Sciences*  
*Texas A&M University*  
*College Station, TX 77843-2133, U.S.*  
*l-lombardini@tamu.edu*

*David N. Appel*  
*Department of Plant Pathology & Microbiology*  
*Texas A&M University*  
*College Station, TX 77843-2132, U.S.*  
*appel@ag.tamu.edu*

**Résumé.** Cette étude évalue les effets des injections d'hydrates de carbone sur la croissance et la vitalité de chênes verts (*Quercus virginiana* P. Miller). Des injections de glucose, de sucrose ou d'un mélange 50:50 de ces deux hydrates de carbone à des concentrations de 0, 40, 80 et 120 g/L ont été faites dans les chênes verts. La croissance du tronc et des racines, la photosynthèse nette, la concentration en hydrate de carbone dans les racines et les pousses, et la fluorescence de la chlorophylle ont été suivis et mesurés. La composition en isotopes d'échantillons de pousses et de racines a été mesurée en tant qu'indicateur de la distribution des hydrates de carbone injectés. Il y avait des différences significatives ( $P < 0,05$ ) dans la croissance du tronc parmi les différents types d'hydrates de carbone, mais aucune différence significative dans la concentrations en hydrates de carbone. Les mélanges de sucrose et de glucose avaient les effets les plus importants comparés à ceux avec un seul sucre, ce qui suggère que le glucose et le sucrose seuls sont utilisés dans des processus autres que celui de la croissance du tronc. Les mélanges 50:50 résultaient en un plus grand effet sur l'ensemble des indices de croissance que les sucres pris seuls. Les contenus en glucose des pousses et de l'amidon dans les racines étaient significativement différents ( $P < 0,05$ ) parmi toutes les moyennes de concentrations avec des niveaux accrus observés chez les arbres traités avec les plus grandes concentrations. La fluorescence de la chlorophylle Fv/Fm a révélé des différences significatives ( $P < 0,001$ ) à toutes les concentrations. Les valeurs d'isotopes de carbone n'ont pas permis de révéler une tendance définitive qui corroborerait la distribution exogène des hydrates de carbone. Les résultats de cette expérience suggèrent que les injections d'hydrates de carbone dans le tronc peuvent avoir un impact sur la croissance et la vitalité du chêne vert.

**Zusammenfassung.** Diese Studie bewertet die Effekte von Kohlenhydrat-Injektionen auf das Wachstum und die Vitalität von Lebensachsen. Glukose, Sukrose oder eine 50:50 Mischung aus beiden Kohlenhydraten mit ansteigenden Konzentrationen (0, 40, 80 und 120 g/L) wurden in die Lebensachsen injiziert. Stamm- und Wurzelwachstum, Nettphotosynthese, Wurzel- und Zweigkohlenhydratkonzentration und die Chlorophyllfluoreszenz wurden überwacht. Die Isotopenzusammenstellung von Zweig- und Wurzelproben wurde als Indikator der Verteilung der injizierten Kohlenhydrate gemessen. Es gab signifikante Unterschiede ( $P < 0,05$ ) im Stammwachstum bei den unterschiedlichen Kohlehydraten, aber keine signifikanten Unterschiede bei den Konzentrationen. Die Mis-

chung von Sukrose und Glukose hatte den größten Wachstumseffekt im Vergleich zu den Einzelzuckern, was schließen lässt, die Einzelzucker eher in anderen Prozessen als Wachstum zu verwenden. 50:50 Mischungen verursachten einen größeren Effekt auf das Durchschnittswachstum als Einzelzucker. Der Glukosegehalt in Zweigen und Stärke in Wurzeln waren deutlich unterschiedlich ( $p < 0,05$ ), während steigende Raten in Bäumen, die mit der höchsten Konzentration behandelt wurden. Die Chlorophyllfluoreszenz Fv/Fm enthüllte hochsignifikante Unterschiede ( $p < 0,001$ ) bei allen Konzentrationen. Die Karbonisotopenwerte zeigten keinen definitiven Trend, welcher mit der exogenen Kohlenhydratkonzentration zusammenhängt. Die Ergebnisse dieses Experiments zeigen, dass Stamminjektionen mit Kohlenhydraten einen Einfluss auf das Wachstum und Vitalität von Lebensachsen haben.

**Resumen.** Este estudio evaluó los efectos de inyecciones de carbohidratos en el crecimiento y vitalidad de encinos siempreverdes (*Quercus virginiana* P. Miller). Se inyectó en encinos glucosa, sucrosa, o una mezcla 50:50 de los dos carbohidratos a concentraciones [0, 40, 80, y 120 g/L (0, 5.3, 10.6, y 16.0 oz/gal)]. Se monitoreó el crecimiento del tronco y raíz, fotosíntesis neta, concentraciones de carbohidratos en raíz y brotes y fluorescencia de clorofila. Se midió la composición de isótopos de tallos y muestras de raíz como un indicador de la distribución de los carbohidratos inyectados. Hubo diferencias significativas ( $P < 0,05$ ) en el crecimiento del tronco entre los tipos de carbohidratos, pero no fueron significativas para las concentraciones de carbohidratos. Las mezclas de sucrosa y glucosa tuvieron el efecto más grande en el crecimiento comparado con solamente azúcar, sugiriendo que la glucosa y la sucrosa solas fueron usadas en procesos diferentes al crecimiento del tronco. Las mezclas 50:50 causaron un mayor efecto en los índices medios de crecimiento que solamente el azúcar. El contenido de glucosa en los tallos y almidón en raíces fue significativamente diferente ( $P < 0,05$ ) entre las medias para las concentraciones con niveles altos encontrados en árboles tratados con las concentraciones más altas. La fluorescencia de clorofila Fv/Fm reveló diferencias altamente significativas ( $P < 0,001$ ) entre las concentraciones promedio. Los valores de los isótopos de carbono no revelaron una tendencia definida que corroborara la distribución exógena de carbohidratos. Los resultados de este experimento sugieren que las inyecciones al tronco de carbohidratos puede tener un impacto en el crecimiento y vitalidad de un encino.