Chapter 3

Determination of the Absorption and Translocation of Exogenous $^{13}\text{C}$-Fructose Applications to Supina Bluegrass under Reduced Light Conditions.

ABSTRACT

Turfves subjected to shady conditions have reduced rates of photosynthesis. This lack of photosynthesis results in lower carbohydrate production, which is a major component for turfgrass growth and development. Turfgrass managers in any discipline often have to deal with shady turf conditions; therefore, investigations to counter this dilemma are warranted. Supplementing low carbohydrate reserves by external carbohydrate applications is one way to potentially compensate for the effects of low light conditions. The objective of this study was to demonstrate the uptake and translocation of exogenous fructose applications to turfgrass grown under reduced light conditions. Results using D-Fructose-$^{13}\text{C}6$ with a surfactant in solution determined that exogenous applications to the leaves of supina bluegrass (Poa supina Schrad.) is readily absorbed and translocated to the crown and roots.

These experiments were a series of investigations that showed how an exogenous fructose application is a potential source of energy if carbohydrate reserves are limiting due to decreased photosynthesis in the shade. Evidence also suggests that weekly fructose applications are sufficient for providing carbohydrate loading in order to compensate for reductions as a result of reduced photosynthesis.
INTRODUCTION

Turves subjected to shady conditions have reduced rates of photosynthesis. This lack in photosynthesis results in lower carbohydrate production, which is a major component of turfgrass growth and development. Turfgrass managers in any discipline (landscape, golf course, or athletic fields) often have to deal with shady turf conditions; therefore, investigations to counter this dilemma are warranted. Supplementing low carbohydrate reserves by external sugar applications is one way to potentially compensate for the effects of low light conditions.

Fructans have been identified as the most common and most important storage carbohydrate in cool season turfgrasses (Hendry, 1987; Hendry, 1993). When plant carbohydrates are limiting, often due to insufficient light causing inhibition of photosynthesis, exogenous applications of sugars have shown potential to compensate for the decrease in carbohydrate synthesis (Berrie, 1960; and Chapter one unpublished research). Research investigating exogenous sugar applications was done to solve a myriad of problems. Exogenous sucrose applications (10% in solution) were found to cause an increase in the dry weight of tomato plants (*Lycopersicum esculentum* Mill.) particularly in conditions where carbohydrate synthesis is limited, and especially when respiration is proceeding rapidly and photosynthesis slowly (Went, 1944; Went and Carter, 1948; Berrie, 1960).
Other exogenous sugar absorption research has looked at plant uptake using leaf disks. Using *Atropa belladonna* leaf disks, Weatherley (1954) attempted to determine that sucrose absorption was an active transport since the water was absorbed passively. However, under aerobic and anaerobic conditions, evidence for the active uptake of sucrose is far from conclusive. Using carrot leaf disks, Grant and Beevers (1963) determined the optimal absorption times and conditions for several sugars. The greatest time for sugar absorption was when the disks were respiring and, specifically for glucose, uptake increased ten fold when temperatures increased from 3 to 25 °C. This study also found that withholding oxygen depressed the uptake of glucose, fructose, galactose and xylose.

Contrasting results have been found when exogenous applications of sucrose have been applied to plants as a spray mixed with urea (46-0-0). Eaton and Ergle (1952) found that spraying the upper leaf surface of cotton plants (*Gossypium hirsutum* var. Missdel x Acala) daily through the fruiting period with 20% sucrose, 1% nitrogen (urea), and the two in combination failed to improve plant growth and resulted in significant decreases in the number of bolls that were set. However, Alvim (1960) determined that 10% sucrose and gibberellic acid were effective in protecting against injury by 2% urea spray on kidney bean (*Phaseolus vulgaris*) plants.

In his research on clover (*Trifolium repens* L.), Van Schreven (1959) found that concentrations of 0.5 and 1% glucose stimulated nodule formation, both in the absence, and in the presence, of additional light. The highest numbers of
nODULES WERE FORMED IN THE PRESENCE OF 2% GLUCOSE. VAN SCHREVEN ALSO FOUND THAT IN THE ABSENCE OF ADDITIONAL LIGHT, NODULATION WAS STIMULATED BY 0.5, 1, AND 2% SUCROSE AND IN THE PRESENCE OF ADDITIONAL LIGHT; NODULATION WAS STIMULATED BY 0.5 AND 1% SUCROSE. IN BOTH CASES, 0.5% SUCROSE CAUSED THE GREATEST STIMULATION.

Exogenous sugar applications for plants in light and dark conditions have also been studied using soybean (Glycine max) seedlings. In the light, translocation of sucrose-\( \text{C}^{14} \) or glucose-\( \text{C}^{14} \) out of the treated leaf was very slow. Only 1% of the \( \text{C}^{14} \) from sucrose was translocated after 14 hours. In the dark, 10% of the \( \text{C}^{14} \) from glucose was translocated to all parts of the seedling in 3 hours. The bulk of the translocated \( \text{C}^{14} \) accumulated in the stem between the treated leaf and the root (Nelson and Gorham, 1957). Although this research was conducted on a legume, the findings on the effects of sugar applications in the light versus dark justify the studying of the effects of sugar applications to turfgrass under reduced light conditions.

While most of the exogenous fructose application research has focused on non-grass (Poacea) crops, some research has looked at the grass family. Exogenous applications to corn (Zea maize) roots concluded that entering glucose and fructose mixed readily with the endogenous pools (Grant and Beevers, 1963). Sucrose uptake also appears to depend on extracellular hydrolysis in young corn roots (Giaquinta, et al., 1983; Lin, et al., 1984; Singh and MacLachlan, 1986). Unfortunately, because turfgrass grows in a contiguous community, the practicality of the applied sugars reaching the roots before
interception from shoots, thatch, soil, insects and microbes is not likely. However, if foliar applications of fructose to the leaves of turfgrass can be absorbed and translocated to the roots, evidence supports that the exogenous sugar will be used as an energy source (Grant and Beevers, 1963).

Compositions incorporating a postemergence herbicide and a sugar, particularly fructose, as a potentiator of the herbicide against weeds without decreasing the tolerance of a crop plant to the herbicide has become a new method for killing weeds. Fructose in solution can be used for plant uptake at a range of 1.25 – 11% weight per volume, but 1.25% is best (Penner and Roggenbuck, 1999). Although this research is focusing on the use of exogenous fructose applications to increase the efficacy of herbicides, subsequent findings have determined that the leaves of both broadleaf and grassy weeds readily take up exogenous fructose applications. However, in order for the fructose to be readily absorbed an organosilicone is necessary as an adjuvant (Penner and Roggenbuck, 1999).

Based on the aforementioned findings for exogenous sugar applications to plants, an investigation is warranted to determine if exogenous sugar applications can be taken up to counter the effects of reduced light conditions (shade) for turfgrass. In this experiment, our objective was to determine whether exogenous applications of fructose (D-Fructose-$^{13}$C$_6$ 99 Atom %, Isotec Inc., Miamisburg, OH, USA) to the leaves of turfgrass under reduced light conditions would be absorbed and translocated to the crown and roots.
Supina bluegrass (Poa supina Schrad.) plots were established from seed on 17 November 2000 on a sand root zone in the indoor research facility (IRF) at the Hancock Turfgrass Research Center at Michigan State University, East Lansing, MI, USA. The indoor research facility is a 400 m$^2$ air-supported structure constructed of Ultralux® (Chemical Fabrics Corporation, Buffalo, NY, USA), a fiberglass fabric which transmits approximately 20% +/- 2% photosynthetically active radiation (PAR; Figure 11). Temperature, relative humidity, and light levels were recorded hourly during the treatment application until the last sampling date using a Spectrum Watchdog Data Logger Model 450 (Spectrum Technologies Inc., Plainfield, Illinois, USA).

On 11 May 2001 whole plants of the established supina bluegrass were transplanted into individual 12.5 cm$^2$ conetainers filled with sand. There were approximately 15 – 18 plants per conetainer. A stock solution was made containing 0.25 g D-Fructose-$^{13}$C$_6$ 99 Atom %, 0.01 ml Sylgard 309 surfactant (Dow Corning, Midland, MI, USA), and 20 ml of double distilled water. Exogenous fructose (D-Fructose-$^{13}$C$_6$ 99 Atom %, Isotec Inc., Miamisburg, OH, USA) applications were initiated on 10 September 2001. Applications were applied to the leaves of the supina bluegrass in the containers as drops using a 1-ml syringe with 0.05-ml solution per treatment.
Figure 11. Photosynthetic photon flux density of sunlight and ambient light inside the indoor research facility (20% +/− 3% transmittance), East Lansing, MI, 48824, 1430 h, 19 June 2000.
**Study one**

Treatments in study one included 0, 1, and 5 fructose applications sampled over time with three replications per treatment. Whole plant samples were collected at 0 (control), 2 (1 apps.), 3 (2 apps.), and 6 (5 apps.) days after treatment. After collection, plant samples were triple rinsed with reverse osmosis water and separated into three parts – shoots, crown, and roots (Figure 12). The shoots consisted of all green plant material; where, the crown area was the compressed stem area and any junction from the first and last node; and finally, the roots were the remaining plant tissue growing below ground. The samples were stored in a freezer at -80 °C, and then freeze dried for 24 hours. Samples were then ground into a fine powder using a mortar filled with liquid nitrogen.

A portion of each sample (1.3-1.5 mg) was then prepared for mass spectrometric analysis on an automated ANCA mass spectrophotometer to evaluate $^{13}\text{C}$ enrichment. Stable C isotope ratios were determined and correction factors applied according to Craig (1957), and the results are expressed as

$$\delta^{13}\text{C}_{\text{PDB}} = \left[ \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right] \times 10^3$$

where $R$ is the $^{13}\text{C}/^{12}\text{C}$, PDB = 0.0112372.

In addition to the $\delta^{13}\text{C}_{\text{PDB}}$ value, the absolute ratio (R), fractional abundance (F), and atom % were other indices used to determine stable isotope abundance from the $^{13}\text{C}$ enrichment. Absolute ratio, fractional abundance and atom % rearrange $\delta^{13}\text{C}_{\text{PDB}}$ in order to calculate the amount of $^{13}\text{C}$ in plant tissues (Boutton, 1991, and Svejcar, et al., 1985).
Figure 12. Illustration of turfgrass plant tissue shoot - crown - root separation.
The absolute ratio of a sample is defined by rearrangement of Eq. 1 as:

\[
R_{\text{sample}} = \frac{^{13}\text{C}}{^{12}\text{C}} = \left(\frac{\delta^{13}\text{C}}{1000} + 1\right) \times R_{\text{PDB}}
\]

Eq. 2.

The fractional abundance is related to \( R \) by the equation:

\[
F = \frac{^{13}\text{C}}{^{13}\text{C} + ^{12}\text{C}} = \frac{R}{R + 1}
\]

Eq. 3.

Atom % is used to express isotopic enrichment in samples highly enriched in \(^{13}\text{C}:

\[
\text{Atom} \% = F \times 100
\]

Eq. 4.

**Study two**

Treatments consisted of consecutive five-time fructose applications with plant samples being collected at 0, 1, and 8 days after application. After collection, plant samples were triple rinsed in double distilled water and separated into three parts - shoots, crown, and roots. The samples were stored in a freezer at -80 °C, and then freeze dried for 24 hours. Samples were then ground into a fine powder using liquid nitrogen. A portion of each sample was then prepared for mass spectrometric analysis and the stable C isotope was calculated exactly to the aforementioned equations.
Study three

The third study was an amendment of study two and was initiated on 30 November 2001. Because the objective of the research was to determine whether exogenous fructose applications can be absorbed by the shoots and translocated to the crown and roots, the study investigating multiple applications was excessive; thus, omitted from being repeated a second time. Instead, a one time fructose application was done with plant sampling being collected at 0, 1, 5, 13, and 20 days after application. Sample collection, preparation, and analysis were done exactly to the aforementioned methods.

Statistical analysis for studies one through three was performed using Agriculture Research Manager version 6.18 (Gylling Data Management, Inc., Brookings, SD, USA). For the duration of the experiments the turfgrass was not mown, and water was applied as a light mist to the leaves daily. Water was also applied directly to the soil every other day using a syringe.
RESULTS AND DISCUSSION

Study one

Experiment one demonstrated significant differences between plant tissues after receiving different frequencies of exogenous fructose applications (Table 4). Fructose applications significantly increased the atom % \((^{13}\text{C}/^{12}\text{C})\) in the turfgrass shoots and crown tissue, regardless of the number of applications suggesting that exogenous fructose applications are readily absorbed and translocated throughout the plant (Figure 13). However, in the shoots, atom % \((^{13}\text{C}/^{12}\text{C})\) was greatest for both one and five fructose applications in the shoots suggesting the shoots as the primary plant tissue for \(^{13}\text{C}\) structural synthesis.

One and five applications of fructose also increased the atom % \((^{13}\text{C}/^{12}\text{C})\) in the roots. The significant decrease in atom % between one and five fructose applications suggests that five days with successive fructose applications is efficiently used, and in turn, is likely lost by respiration and/or root exudation.
Table 4. Study one analysis of variance of plant tissue over time for $^{13}$C Atom % ($^{13}$C/$^{12}$C) from exogenous $^{13}$C-fructose applications to supina bluegrass under reduced light conditions. E. Lansing, MI. 48824. 10 – 19 Sep. 2001.

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* Significant at the 0.05 probability level.

† Tissue consists of shoot – crown – root separation.

‡ Time consisted of sampling a control treatment, and sampling one day after one and five days of exogenous fructose applications.
Figure 13. Plant tissue (shoots:crown:roots) over time atom % (\(^{13}\text{C}/^{12}\text{C}\)) interaction for consecutive \(^{13}\text{C}\)-fructose applications to supina bluegrass under reduced light conditions. East Lansing, MI. 48824. 10 - 19 September 2001.
Study two

No significant differences occurred between plant tissues over time when plant tissue sampling was done at 0, 1, 8, and 13 days after applying fructose for five consecutive days (Table 5). Fructose applications significantly increased the atom % ($^{13}$C/$^{12}$C) in the turfgrass shoots and crown tissue one day after receiving five consecutive days of exogenous fructose applications. This result shows that the fructose is readily absorbed and translocated throughout the plant (Figure 14). One day after receiving five consecutive days of exogenous fructose applications also increased the atom % ($^{13}$C/$^{12}$C) in the roots.

Net $^{13}$C (atom %) accumulation in the crown and roots occurred eight days after the last application (Figure 14). At the same time, a significant decrease in $^{13}$C (atom %) accumulation was occurring between the shoots for the one and eight day sampling times. After eight days, the continuous increase in $^{13}$C (atom %) accumulation in the crown and roots coupled with the decrease in $^{13}$C (atom %) accumulation for the shoots suggests a source to sink relationship occurring. Furthermore, continued fructose absorption may be occurring through the leaf sheath near the crown. These results also suggest that the exogenous fructose is being absorbed and translocated to the crown and the roots for storage, as if it were synthesizing carbohydrates via photosynthesis. In addition, sometime between the eight and 13 day sampling time, $^{13}$C (atom %) accumulation began to decrease in the roots and increase in the shoots, suggesting the movement of isotope $^{13}$C to the shoots from the roots for structural synthesis. These results suggest that not only are exogenous fructose applications being readily absorbed
and translocated, but that the exogenous fructose is becoming part of the endogenous carbohydrate pool and is being used for the plants metabolic processes.
Table 5. Study two analysis of variance of plant tissue over time for \(^{13}\)C Atom \(^{\%}\) \((^{13}\text{C}/^{12}\text{C})\) from exogenous \(^{13}\)C-fructose applications to supina bluegrass under reduced light conditions. E. Lansing, MI. 48824. 10 – 27 Sep. 2001.

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* Significant to the 0.05 probability level.

\(^\dagger\) Tissue consists of shoot – crown – root separation.

\(^\dagger\) Time consisted of sampling a control treatment, and samples collected 1, 8, and 13 days after five days of exogenous fructose applications.
Figure 14. Atom % ($^{13}$C/$^{12}$C) significance over time with source sink relationship on supina bluegrass plant tissue (shoots:crown:roots) after five days of exogenous $^{13}$C-fructose applications. East Lansing, MI. 48824. 10-27 September 2001.
Study three

Similar to study one, study three demonstrated significant differences between plant tissue and time (Table 6). Fructose applications significantly increased the atom % ($^{13}$C/$^{12}$C) in the turfgrass shoots, crown, and root tissue at all four sampling dates after receiving an exogenous fructose application. This result suggests that fructose applications are readily absorbed and translocated throughout the plant (Figure 15). Net $^{13}$C (atom %) accumulation in the shoots and crown tissue occurred for the first three sampling dates after the fructose application (1, 5, and 13 days). Similar increases occurred in the roots through the first two sampling dates after applying the fructose. However, between 13 and 20 days after receiving fructose, a significant decrease in atom % ($^{13}$C/$^{12}$C) occurred in the crown tissue.

Results from this experiment suggest that even with one fructose application, fructose absorption continues over time, likely through the sheath at the crown area (Figure 15). In addition, net atom % ($^{13}$C/$^{12}$C) accumulation is occurring in the roots with only one exogenous fructose application and, over time, the fructose is being used metabolically for structural synthesis and respiration. The continued increase in atom % ($^{13}$C/$^{12}$C) several days after the exogenous fructose application also suggests that the preexisting $^{12}$C-carbohydrate sources are being metabolized and respired.
Table 6. Study three analysis of variance of plant tissue over time for $^{13}$C Atom % ($^{13}$C/$^{12}$C) from exogenous $^{13}$C-fructose applications to supina bluegrass under reduced light conditions. E. Lansing, MI. 48824. 30 Nov – 21 Dec. 2001.

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* Significant at the 0.05 probability level.

† Tissue consists of shoot – crown – root separation.

‡ Time consisted of sampling a control treatment, and samples collected 1, 5, 13, and 20 days after one exogenous fructose applications.
Figure 15. Plant tissue (shoots:crown:roots) over time atom % (\(^{13}\text{C}/^{12}\text{C}\)) interaction on supina bluegrass under reduced light conditions after one exogenous \(^{13}\text{C}\)-fructose application. East Lansing, MI. 48824. 30 November - 21 December 2001.
Comparing the results of study one and two versus the findings from study three suggests that five days of exogenous fructose applications appears to be vain. The results of the three studies determined that after an exogenous fructose application ($^{13}$C-fructose), significant reductions in $^{13}$C do not begin to occur until after 13 days. These findings support the findings from chapter one, where it was determined that weekly fructose applications provided a higher turfgrass quality than applications applied five times per week.
CONCLUSIONS

Exogenous fructose applications to the leaves of turves grown under low light conditions were successfully taken up and translocated to the shoots, crown, and roots. Evidence suggests that exogenous fructose is mixing with the endogenous carbohydrate pool and is being used for metabolic processes. These experiments were a series of investigations that showed how an exogenous fructose application is a potential source of energy if carbohydrate reserves are limiting due to decreased photosynthesis in the shade. Evidence also suggests that weekly fructose applications are sufficient for providing carbohydrate loading in order to compensate for reductions as a result of reduced photosynthesis.
REFERENCES


