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Sugar in Shade: The Effects of Exogenous Fructose Applications to Turfgrass under Reduced Light Conditions

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SUGAR IN SHADE: THE EFFECTS OF EXOGENOUS FRUCTOSE APPLICATIONS TO TURFGRASS UNDER REDUCED LIGHT CONDITIONS

By

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ABSTRACT

Sugar in Shade: the Effects of Exogenous Fructose Applications to Turfgrass under Reduced Light Conditions.

By

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Turves 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 profession often have to deal with shady turf conditions; therefore, investigations to counter this dilemma are warranted. Supplementing low carbohydrate reserves by exogenous carbohydrate applications is one way to potentially compensate for the effects of low light conditions. The objective of this research was to: 1) determine whether a physiological response occurs with exogenous fructose applications to turfgrass grown under reduced light conditions, 2) demonstrate the uptake and translocation of exogenous fructose applications to turfgrass grown under reduced light conditions, and 3) compare photosynthetic carbon partitioning between cool-season turfgrass species grown under full sun and reduced light conditions.

For supina bluegrass growing under reduced light conditions the rate of 1.25% weight per volume fructose applied at 1, 2, 4, 6, and 8 times 815-L ha^{-1} is acceptable in terms of injury and triggers positive physiological changes to

occur compared to the control plants where no fructose applications were applied. Furthermore, it was found that 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. The results also suggest that the exogenous fructose is mixing with the endogenous carbohydrate pool and is being used for metabolic processes.

Discoveries from this research will provide valuable insight for the future management of turfgrass under reduced light conditions.

DEDICATED

To my fiancé Lisa M. Lundberg,

And my parents Shirley Jones & Sam Sorochan

Thank you

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INTRODUCTION

Turfgrass is used for athletics, recreation, aesthetics, and utility. Each of these situations requires turf to perform a different role in a different setting. As a result of this dynamic utilization, turfgrass is often managed under sub-optimal growing conditions. One such condition is shade (reduced light conditions; RLC). Turfgrass located in RLC makes up about 20-25% of all managed turfgrass (Beard, 1973).

Turfgrass management in RLC (<30% full sunlight) is difficult because turf growth is affected by a lack of sufficient light energy (Stier, 1997). Surrounding plant material, clouds, or other shading structures can reduce the light intensity by as much as 95% (Beard, 1973). As a result, the light quality that reaches the turf surface is altered, thereby creating an environment non-conducive to normal plant growth and development (Wilson, 1997).

Sunlight provides energy and supports the base of the food chain for all life on earth. Autotrophs utilize light energy from the sun for photosynthesis by oxidizing water and reducing CO_2 into organic compounds, thus providing energy for growth, development and reproduction (Taiz and Zeiger, 1991).

$$Energy_{light} + 24H_2O + 12CO_2 \longrightarrow 12O_2 + C_{12}H_{24}O_{12} + 12H_2O$$

Photosynthesis is driven by the visible portion of the light spectrum (400 to 700 nm), called photosynthetically active radiation (PAR; Lawlor, 1987), and compromises up to 50% of the earth's direct radiation (Salisbury and Ross, 1992).

Plants only utilize 1 to 5% of the annual available light and 3 to 10% of the available light during maximum growth for photosynthesis (Cooper, 1970); therefore, it is important to consider the amount of PAR reaching the plants, particularly in RLC. The light compensation point is defined as the light level where net photosynthesis is zero or where the photosynthesis rate equals the respiration rate (Danneberger, 1993). Plant survival requires net photosynthesis to exceed the rate of respiration (Wilkinson *et al.*, 1975). In RLC, light levels are often below the compensation point resulting in the exhaustion of carbohydrate reserves for respiration. The carbohydrate fixation pathway (Calvin cycle) for cool season turfgrass only requires 116 to 233 W m^{-2} of light compared to over 900 W m^{-2} for warm season turfgrasses found in temperate regions on a clear solar noon (Dudeck and Peacock, 1992).

Turf Responses to Shade

Lower light intensity, altered light quality, and change in microclimate produce many physiological, morphological, and anatomical responses in turfgrass species (Beard 1973; Dudeck and Peacock, 1992; Danneberger, 1993; Wilson, 1997). The physiological responses to RLC are mostly attributed to the change in red/far-red ratios of light (Wilson, 1997). Turfgrass plants become more spindly with increased shoot elongation, decreased density and tillering, decreased shoot width, thicker leaves, reduced in appearance of successive leaves on stem and increased overall plant height (Beard 1973; Dudeck and Peacock, 1992; Danneberger, 1993; Wilson, 1997). Photosynthesis and respiration are both reduced under RLC (Dudeck and Peacock, 1992). Other

physiological responses include lower CO_2 compensation point, fewer carbohydrate reserves, reduced carbon/nitrogen ratio, decreased transpiration rate due to lower osmotic pressure and increased tissue moisture, higher lignin content, and reduced flowering (Beard, 1973).

Anatomical and morphological changes in turfgrass can be attributed to both the change in environment and the change in light quality. Anatomically, shaded turf has decreased chloroplasts and increased thylakoid and grana stack development, (this could be attributed to a shift towards better photosynthetic efficiency). In addition, turf under RLC has thinner cuticles, decreased stomatal density, and less vascular tissue due to the restricted air movement and higher relative humidity. Morphologically, turfgrass leaves become thinner and longer with an increased overall leaf area, and the root/shoot ratio is increased due to the response of vertical growth from increased shoot length. There is also a decrease in stem diameter, internode and stolon number and length, shoot density, and rhizome growth, horizontal growth habit, and dry weight material. There is an increase in spongy parenchyma cells, vertical growth habit, and succulence (Beard, 1973; Dudeck and Peacock, 1992; Danneberger, 1993; Wilson, 1997).

Cultural Solutions and previous research

Beard (1973) suggests several cultural practices to maximize the quality of turf found under RLC. First, the mowing height should be raised to maximize the light interception by shoots and depth of roots. Second, irrigation should be applied judiciously. This causes an increase in relative humidity with lower

stomata density, and thinner leaves. However, additional irrigation can be detrimental to the weak turf and would provide a more favorable environment for disease, moss and algae invasion. Third, high levels of nitrogen should be avoided. Nitrogen decreases the root/shoot ratio, while nitrogen assimilation is competitive for resources used in respiration (Powell, Blaser and Schmidt, 1967; Schmidt and Blaser, 1967; Green and Beard, 1969; Beard, 1973; Mazuer and Hughes, 1976; Westhafer, Law and Duff, 1982). Higher levels of nitrogen can also increase disease incidence and severity (Goss and Gould, 1967; Markland, Roberts and Frederick, 1969). Fourth, use shade tolerant species where appropriate. Certain species have the ability to tolerate the conditions found with reduced light. Cultivars within a species can also differ in shade tolerance (Dudeck and Peacock, 1992). Fifth, an integrated pest management strategy should be employed to combat the higher disease pressure. Sixth, traffic should be reduced to prevent further damage to the weak, spindly turf. Seventh, tree limbs and roots should be trimmed to minimize competition for light, nutrients, water, and other resources. Finally, plant growth regulators can be utilized to alter the growth habit to more favorable characteristics (Goss, *et al.*, 2002).

Fructan knowledge

Turves subjected to RLC have reduced rates of photosynthesis. This lack of photosynthesis results in lower carbohydrate production, which is a major component for turfgrass growth and development. The majority of non-structural carbohydrates found in turfgrass are glucose, fructose, sucrose, various

oligosaccharides, starch and short- and long-chained fructans (Hull, 1992; Smith, 1972; Westhafer *et al.*, 1982). Small and short-chained sugars are utilized rapidly for growth and maintenance of plant tissues, while starch and longer-chained fructans are utilized for storage. Warm season grasses accumulate starch or sucrose, while cool season grasses accumulate mostly fructose polymers (fructans) with some starch and sucrose (Smith, 1968; Smith, 1972). The primary turfgrass seed storage carbohydrate is starch (Beard, 1973). The early photosynthetic products consist of sucrose and/or starch depending on the immediate energy requirements of the plant cells and the transporting ability of the plant. Glucose, fructose and sucrose levels are high in root and leaf tissue compared to the storage organs of stems, rhizomes and stolons (Beard, 1973). Fructans, which are synthesized from sucrose as a result of carbohydrate production, have been identified as the most common and most important sugars in grasses (Edelman and Jefford, 1968; Meier and Reid, 1982; Hendry, 1987; Chatterton *et al.*, 1989; Smouter and Simpson, 1989; Pollock and Cairns, 1991).

Perennial grasses in temperate regions are naturally exposed to prolonged periods of low temperatures (chilling temperatures). Fructan is the main polysaccharide reserve in vegetative tissues in most cool season grasses (Pointis and Del Campillo, 1985; Pollock, 1986; Nelson and Spollen, 1987; Chatterton *et al.*, 1989). Generally, low ambient temperatures lead to an alteration in the balance between carbon assimilation and utilization. This results in a pronounced increase in fructan and sucrose contents in the leaves of barley (Wagner and Wiemken, 1989), *Lolium temulentum* (Pollock, 1984) *Lolium*

perenne (Arbillot *et al.*, 1991), *Triticum aestivum* (Tognetti, Calderon and Pontis: 1989), Tognetti *et al.*, 1990) and *Poa pratensis* (Solhaug, 1991). The role of fructans in cold acclimation remains an open question since their accumulation most likely results from sucrose accumulation (Pollack, 1984; Wagner, Wiemken and Matile, 1986) rather than from low temperatures.

Fructan content in roots also varies throughout the season with the highest concentrations occurring in the fall and the minimum in the spring (Steen and Larsson, 1986). However, under controlled conditions, low temperatures increased both sugar and fructan concentrations in roots of *Poa pratensis* (Solhaug, 1991), *Agropyron* and *Agrostis alba* (Chatterton *et al.*, 1987).

Rational

Turfgrass managers in any discipline (landscape, golf course, or athletic fields) often have to deal with RLC; therefore, investigations to counter this dilemma are warranted. Supplementing low carbohydrate reserves by external sugar applications is one potential method to compensate for the effects of RLC.

If turfgrass is growing under sub-optimal light conditions an increase in growth can potentially be affected by exogenous sources of sugar. By spraying tomato plants grown under a variety of conditions, with 10% sucrose solution, it was shown that sugar affected a greater increase in dry weight if the tomato is growing in conditions where carbohydrate synthesis is limited (Wen and Carter, 1948; Juhren and Went, 1949; and Berrie, 1959). Attempts to have fructose taken up by turfgrasses has previously been explored; however, it was

determined that the efficiency of plant uptake from exogenous fructose applications was not prudent (Branham, 1999). This is likely a result of the molecule size of the sucrose being too large for plant cell absorption. Experiments testing the use of fructose for turfgrass uptake have been very successful (Penner and Roggenbuck, 2000). Fructose applications at 1.25% by solution have been shown to greatly increase the efficacy for herbicide control when used in conjunction with an adjuvant (Penner and Roggenbuck, 2000).

There are five specific questions that arise concerning exogenous fructose applications to turfgrass under reduced light conditions. 1) Does the fructose get into the plant? 2) Once in the plant is the fructose translocated and metabolized? 3) Do the exogenous fructose applications yield a positive physiological response? 4) Is this applicable to other turfgrasses including C-3 and C-4 grasses? Finally, 5) What other implications for overcoming limitations for low light exist? With respect to the aforementioned questions, this research will attempt to answer question one and two.

Specific Objectives

1. Determine whether a physiological response occurs with exogenous fructose applications to turfgrass grown under reduced light conditions.
2. Demonstrate the uptake and translocation of exogenous fructose applications to turfgrass grown under reduced light conditions.
3. Compare photosynthetic carbon partitioning between cool-season turfgrass species grown under full sun and reduced light conditions.

Chapter 1

Determination of shade tolerance for three cool-season turfgrass species (Kentucky bluegrass, tufted hairgrass, and tall fescue) using $^{13}CO_2$ pulse chase procedures.

ABSTRACT

Reduced light conditions are said to comprise an estimated 20-25% of all managed turfgrass. To further understand the relative shade tolerance a simple study was initiated using three cool-season turfgrass species. In this experiment, Kentucky bluegrass (*Poa pratensis* L.), tufted hairgrass (*Descampsia caespotosa*), and tall fescue (*Festuca arundianacea* Schreb.) were labeled with $^{13}CO_2$ during photosynthesis to assess C allocation under full sun and reduced light conditions. The objective of this study was to compare photosynthetic carbon partitioning between cool-season turfgrass species grown under full sun and reduced light conditions. Results for this experiment determined that reduced light levels provided by the indoor research facility are not limiting enough for tall fescue and tufted hairgrass, thus suggesting their effective shade tolerance. Because tall fescue and tufted hairgrass are slow growing turfgrasses, the level of reduced light did not limit their photosynthetic requirements. Conversely, the $^{13}CO_2$ pulsing suggested that the shade tolerance of Kentucky bluegrass, a more aggressive growing turfgrass, to be insufficient under the reduced light level.

INTRODUCTION

The use of stable carbon (C) isotopes in agricultural and ecological research has become more frequent in recent years (Tieszen and Boutton, 1989). The two stable isotopes of carbon are ^{12}C and ^{13}C, which comprise 98.89 and 1.11%, respectively, of all C in nature. Early ^{12}C and ^{13}C research utilized natural variation in the relative abundances of these two stable isotopes. For example, cool-season (C_3) plant species discriminate against $^{13}CO_2$ during photosynthesis to a greater extent than do warm-season (C_4) plant species (O'Leary, 1981). This difference in $^{13}C/^{12}C$ ratio between C_3 and C_4 plants has been used to estimate the proportion of C_3 and C_4 species in diets of rangeland insects and large herbivores (Boutton *et al.*, 1980; Jones *et al.*, 1979). The difference in $^{13}C/^{12}C$ ratio has also been studied in root cores containing the two aforementioned functional groups (Svejcar and Boutton, 1985). The C isotope ratios of C_3 plants have also been shown to be correlated with water-use efficiency (Farquhar and Richards, 1984), which makes C isotope analysis a useful tool in ecophysiology and plant breeding research.

In addition to C isotopic studies, which capitalize on natural $^{13}C/^{12}C$ variation, plants can also be labeled with $^{13}CO_2$ during photosynthesis to assess C allocation. Boutton, *et al.* (1987) labeled rice (*Oryza sativa* L.) to obtain grain enriched in ^{13}C for use in human nutrition studies. Mordacq *et al.* (1986) studied C flow to roots and respiratory losses in a chestnut coppice using $^{13}CO_2$. Kouchi and Yoneyama (1984a,b) used a steady state $^{13}CO_2$ assimilation system and

long-term labeling to study accumulation, translocation, and metabolism of photosynthetically assimilated C in nodulated soybean (*Glycine max* L.) plants. The aforementioned ^{13}C tracer experiments implemented in agronomic or ecological context are relatively simple procedures for labeling plants with $^{13}CO_2$ (Svejcar, *et al.*, 1990). While much C labeling using $^{13}CO_2$ has been done, nothing has been done on turfgrass exposed to reduced light conditions (shade).

In this experiment, three cool-season turfgrass species were labeled with $^{13}CO_2$ during photosynthesis to assess C allocation under full sun and reduced light conditions. Reduced light conditions is said to comprise an estimated 20-25% of all managed turfgrass (Beard, 1973). With cool-season turfgrass species, differences in shade adaptation have also been found. Supina bluegrass (*Poa supina* Schrad.) is a cool season turfgrass that has been identified as having exceptional shade adaptation (Berner, 1984; Pietsch, 1989; Stier and Rogers, 2001) while tall fescue (*Festuca arundinacea*), creeping red fescue (*Festuca rubra* L. ssp. r*ubra*), creeping bentgrass (*Agrostis stolonifera* L.), and colonial bentgrass (*Agrostis capillaris* L.) have good shade tolerance. Kentucky bluegrass (*Poa pratensis* L.) has been identified as having poor shade tolerance (Dudeck and Peacock, 1992).

MATERIALS AND METHODS

The accumulation and translocation of photosynthetically assimilated carbon using $^{13}CO_2$ was studied using established stands of Kentucky bluegrass (*Poa pratensis* L.), tufted hairgrass (*Descampsia caespotosa*), and tall fescue (*Festuca arundinacea* Schreb.) grown under full sun and reduced light conditions. The experimental design for this study was a completely randomized factorial design with three replications. On 18 May 2000, 18 (six of each species) 175 cm^2 pots were filled with sand and seeded outside at the Hancock Turfgrass Research Center at Michigan State University, East Lansing, MI. The seeding rates were 5, 10, and 35 grams of seed m^{-2}, for Kentucky bluegrass, tufted hairgrass, and tall fescue, respectively. Fertilizer was applied once every two weeks for the first six weeks using Lebanon Country Club (Lebanon, PA, USA) 13-25-12 Starter Fertilizer at 5 grams N m^{-2}. Beginning 30 June 2000 and continuing through 17 August 2001, 5 grams N m^{-2} were applied every three weeks using Lebanon Country Club 18-3-18 fertilizer. On 6 October 2000 all pots were moved into the indoor research facility at the Hancock Turfgrass Research Center to continue to grow under reduced light conditions. On 28 April 2001 three pots of each species were taken outside to acclimate to full sun conditions.

The indoor research facility at the Hancock Turfgrass Research Center 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 (Figure 1). 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 14 August 2001 $^{13}CO_2$ labeling was done between 0900 h and 1200 h by placing individual turfgrass pots into sealed chambers with a Mylar® (Du Pont, Wilmington, DE, USA) cover for light transmittance. To generate $^{13}CO_2$ for ^{13}C labeling, one ml of $Ba^{13}CO_3$ (98 atom %, Isotec Inc., OH, USA) and one ml of 85% lactic acid (Baker, Phillipsburg, NJ, USA) were mixed using two five ml syringes. Once generated, the $^{13}CO_2$ was injected into the gastight chamber for labeling (Figure 2). The total CO_2 concentration following injection was greater than 900 ppm above ambient when the CO_2 levels were initially measured using a CIRAS-1 infrared gas analyzer (PP-Systems Inc., Haverhill, MA, USA). Because preliminary tests had shown daily photosynthesis to end earlier outside than inside, $^{13}CO_2$ labeling was done to all of the turfgrass species growing outside, then to the pots growing inside.

Plant samples were harvested at 1 and 24-h, and 7 days post $^{13}CO_2$ labeling. In addition, one set of unlabeled plants was harvested to obtain natural ^{13}C abundances. Plant samples were rinsed in double distilled water and separated into crown, roots, and shoots (Figure 3). 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

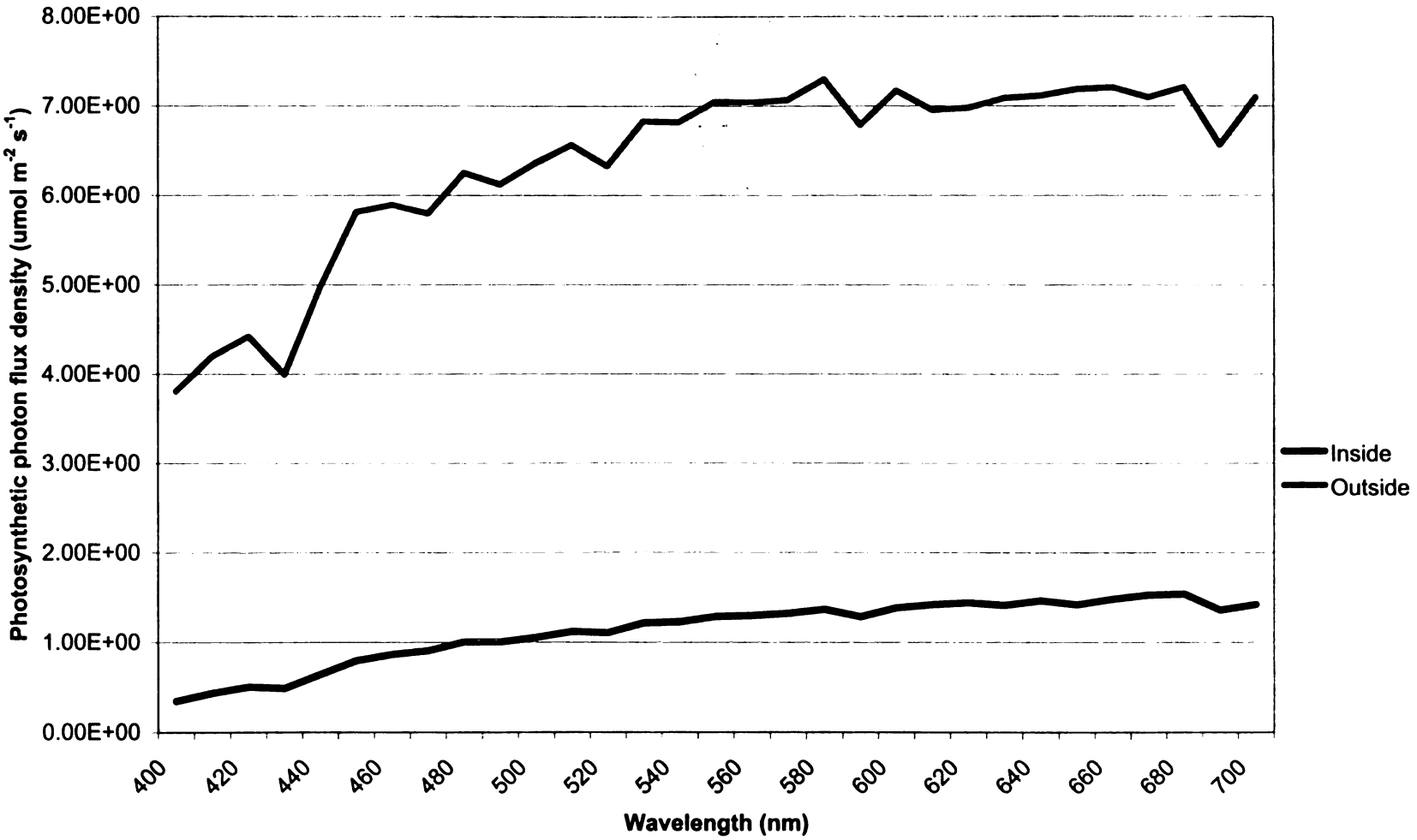

Figure 1. Photosynthetic photon flux density of sunlight and ambient light inside the indoor research facility, East Lansing, MI. 48824. 1430 h, 19 June 2000.

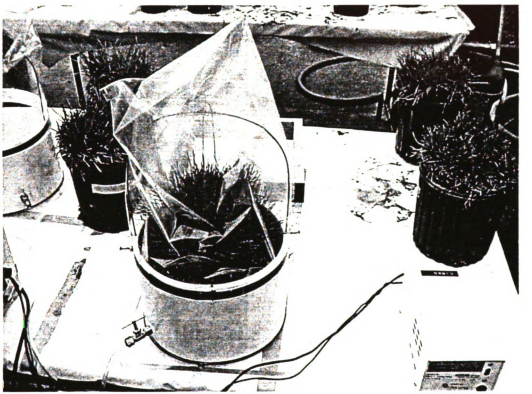

Figure 2. Photograph of closed system for $^{13}CO_2$ pulsing on cool - season turfgrasses. East Lansing, MI. 48824. 14 August 2001.

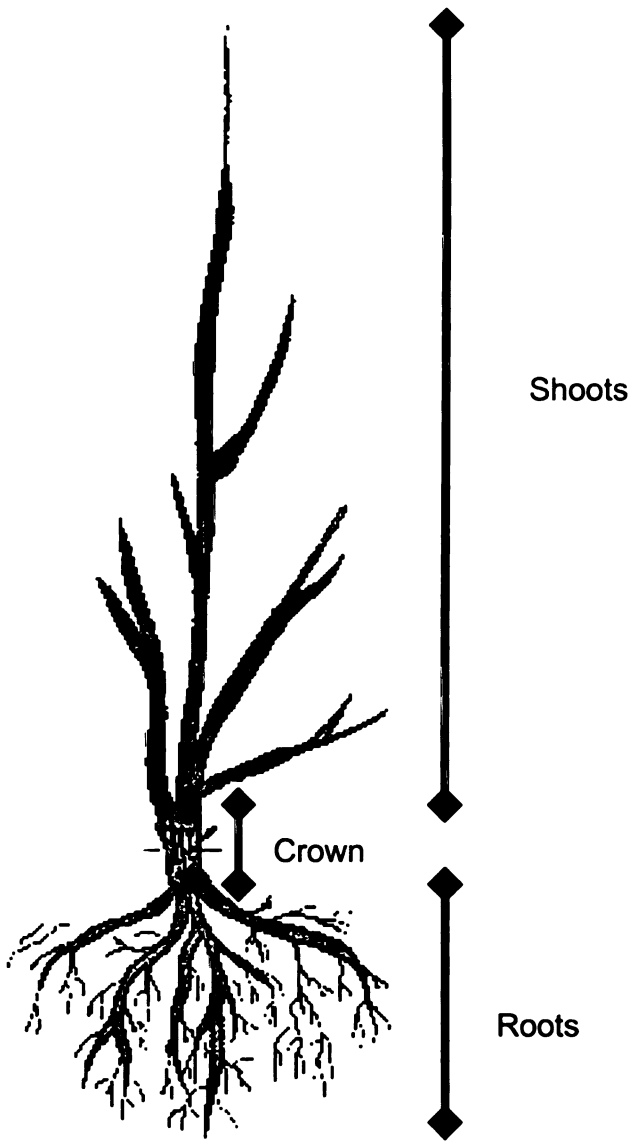

Figure 3. Illustration of turfgrass plant tissue shoot - crown - root separation.

plant tissue growing below ground. Separated plant parts were placed in aluminum foil, frozen in liquid nitrogen, and then transferred to a freezer at -80 °C. Next, the plant samples were put into a freeze drier for 24 hours. After 24 hours, the plant samples were ground into a fine powder using a mortar and 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}C enrichment. Stable C isotope ratios were determined and correction factors applied according to Craig (1957), and the results are expressed as

Eq. 1. $$\delta^{13}C_{PDB} = \left[\frac{R_{sample}}{R_{standard}} - 1 \right] x\ 10^3$$

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

In addition to the $\delta^{13}C_{PDB}$ value, the absolute ratio (R), fractional abundance (F), and atom % were other indices used to determine stable isotope abundance from the ^{13}C enrichment. Absolute ratio, fractional abundance and atom % rearrange $\delta^{13}C_{PDB}$ in order to calculate the amount of ^{13}C in plant tissues (Boutton, 1991, and Svejcar, *et al.*,).

The absolute ratio of a sample is defined by rearrangement of Eq. 1 as:

Eq. 2. $$R_{sample} = {}^{13}C/{}^{12}C = \left[\frac{\delta^{13}C}{1000} + 1 \right] x\ R_{PDB}$$

The fractional abundance is related to R by the equation:

Eq. 3. $$F = \frac{^{13}C}{^{13}C + ^{12}C} = \frac{R}{R + 1}$$

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

Eq. 4. Atom % = F x 100

Statistical analysis was done using Agriculture Research Manager, version 6.18 (Gylling Data Management, Inc. Brookings, SD, USA).

RESULTS AND DISCUSSION

Atom % ($^{13}C/^{12}C$) differences in plant tissue sampling were significantly higher in the shoots versus the crown and roots one hour after $^{13}CO_2$ pulsing for all three species tested. At 168 hours after $^{13}CO_2$ pulsing, only Kentucky bluegrass had significant differences for ^{13}C partitioning (atom %) when comparing the reduced light versus full sun treatments (Table 1). Trends for the regression of ^{13}C partitioning in the shoots of all three species tested (atom % relative to the control) suggests that only Kentucky bluegrass has altered photosynthesis when subjected to reduced light conditions (Figure 4). These differences suggest that the level of reduced light conditions during testing, significantly impacted the photosynthetic process of Kentucky bluegrass, and not the other two species tested (tall fescue and tufted hairgrass).

Evidence suggests that Kentucky bluegrass under reduced light levels is using photosynthetic CO_2 more efficiently for structural synthesis, particularly in the shoots. These findings suggest that Kentucky bluegrass is not tolerant to the reduced light conditions of the indoor research facility; therefore, photosynthate CO_2 is being used for structural growth in the etiolated shoots.

Table 1. Analysis of variance for atom % ($^{13}C/^{12}C$) significance using $^{13}CO_2$ pulse chase technique on three cool-season turfgrasses grown in full sun and reduced light conditions. E. Lansing, MI. 48824. 14 August 2001.

		MSE (1×10^{-4})								
		Kentucky bluegrass			Tall fescue			Tufted hairgrass		
SOURCE	DF	1 hr	48 hrs	168 hrs	1 hr	48 hrs	168 hrs	1 hr	48 hrs	168 hrs
Total	17									
R	2	5	1	6	2	3	3	14	19	8
Tissue† (A)	2	1300*	4	20*	210*	4	5	426*	6	1
Location‡ (B)	1	193*	5	27*	1	9	3	22	2	14
AB	2	142*	1	10	0	5	2	1	0	3
ERROR	10	9	10	3	4	3	2	9	7	4

* Indicates significance at the 0.05 probability level.

† Reduced light conditions were from the indoor research facility with about 20% +/- 3% full sun transmittance.

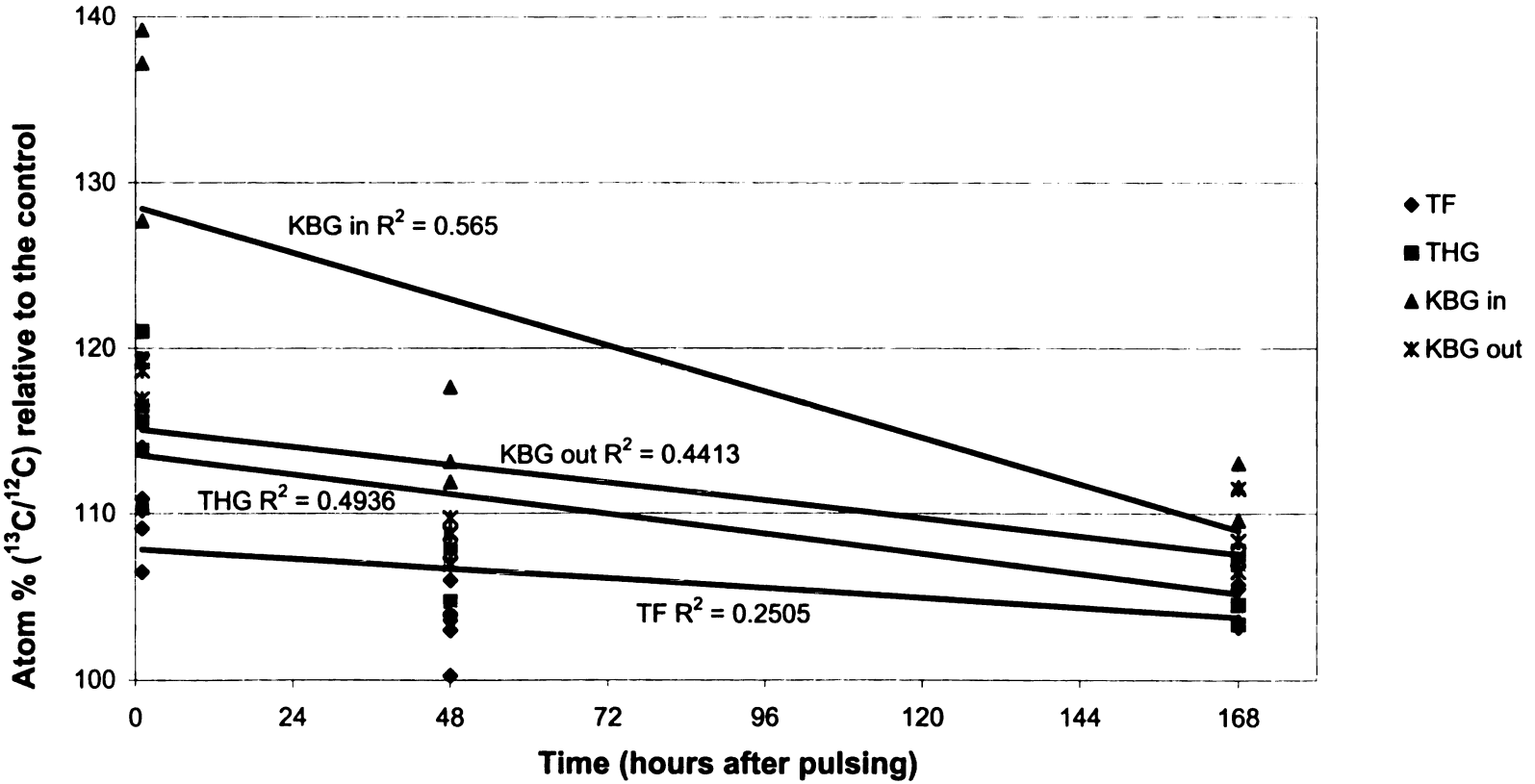

Figure 4. Regression of ^{13}C partitioning, for atom %; $^{13}C/^{12}C$ relative to the control, for the shoots of tall fescue (TF), tufted hairgrass (THG), and Kentucky bluegrass (KBG) after $^{13}CO_2$ pulsing. E. Lanisng, MI. 48824. 14 August 2001.

CONCLUSIONS

The $^{13}CO_2$ pulse chase experiments were done on turfgrasses where no other foreign stresses (traffic, nutrient deficiencies, water stress, etc.) where present. Results for this experiment determined that the reduced light levels provided by the indoor research facility are not limiting enough for tall fescue and tufted hairgrass, thus suggesting their effective shade tolerance. Because tall fescue and tufted hairgrass are slow growing turfgrasses, the level of reduced light did not limit their photosynthetic requirements. Conversely, the $^{13}CO_2$ pulsing suggested that the shade tolerance of Kentucky bluegrass, a more aggressive growing turfgrass, to be insufficient under the reduced light conditions. Therefore, successful determination of turfgrass shade tolerance for each species with respect to the reduced light conditions from the indoor research facility was determined using $^{13}CO_2$ pulse chase experiments.

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Chapter 2

Effects of Exogenous Fructose and Adjuvant Applications on Leaf Injury of Supina Bluegrass under Reduced Light Conditions.

ABSTRACT

Turfgrass management in reduced light conditions (<30% full sunlight) is difficult because turf growth is affected by a lack of sufficient light energy. In turn, plant carbohydrates are reduced, due to insufficient light inhibiting photosynthesis. To combat this problem, a simple study was initiated to investigate the potentials for exogenous fructose applications to the leaves of turfgrass growing under reduced conditions. Thus, the objective of this study was to determine whether a physiological response occurs with exogenous fructose applications to turfgrass grown under reduced light conditions. Fructose applied to supina bluegrass under reduced light conditions five times per week for eight consecutive weeks causes too much leaf injury unless the application rate is 1.25% weight per volume fructose at the 815-L ha^{-1} rate with 0.1% weight per volume Break Thru® adjuvant. Weekly fructose applications (one time per week) yielded acceptable levels of leaf tissue injury for the 1,2,4,6, and 8 times application rates. The 1x rate provided the least amount of leaf tissue injury while demonstrating positive physiological responses compared to the control.

INTRODUCTION

Turfgrass is used for athletics, recreation, aesthetics, and utility. Each of these situations requires turf to perform a different role in a different setting. As a result of this dynamic utilization, turfgrass is often managed under sub-optimal growing conditions. One such condition is shade (reduced light conditions; RLC). Turfgrass located in RLC makes up about 20-25% of all managed turfgrass (Beard, 1973).

Turfgrass management in RLC (<30% full sunlight) is difficult because turf growth is affected by a lack of sufficient light energy (Stier and Rogers, 2001). Light intensity can be reduced by as much as 95% from surrounding plant material, clouds, or other shading structures (Beard, 1973). As a result, the light quality that reaches the turf surface is altered, creating an environment non-conducive to normal plant growth and development (Wilson, 1997).

Plants need light energy from the sun for photosynthesis to provide energy for growth, development, and reproduction (Taiz and Zeiger, 1991).

$$Energy_{light} + 24H_2O + 12CO_2 \longrightarrow 12O_2 + C_{12}H_{24}O_{12} + 12H_2O$$

Photosynthesis is driven by the visible portion of the light spectrum (400 to 700 nm), called photosynthetically active radiation (PAR; Lawlor, 1987), and comprises up to 50% of the earth's direct radiation (Salisbury and Ross, 1992). When plant carbohydrates are limiting, often due to insufficient light from inhibited photosynthesis, exogenous applications of sugars have shown potential to become a management technique for turf under RLC (unpublished data).

Early research has focused on the potential for exogenous sugar applications to be taken up by a myriad of plant species. Exogenous sucrose applications (10% in solution) were found to cause an increase in the dry weight of tomato plants (*Lycopersicum esculentum* Mill., Went, 1944; Went and Carter, 1948). In addition, Berrie (1960) determined a greater increase in tomato dry weight with exogenous sucrose applications (10% in solution) particularly in conditions where carbohydrate synthesis is limited, and especially when respiration is proceeding rapidly and photosynthesis slowly.

Other exogenous sugar absorption research has looked at plant uptake using leaf disks. Weatherley (1954) attempted to determine that sucrose absorption was an active transport when the water was absorbed through passive absorption using *Atropa belladonna* leaf disks. 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 for carrot leaf disks was when the disks were respiring. Glucose uptake also increased ten fold when temperatures increased from 3 – 25 °C. Finally, withholding oxygen depressed the uptake of glucose, fructose, galactose and xylose (Grant and Beevers, 1963).

Mixed 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. In a related study, Alvim (1960) determined that 10% sucrose and gibberellic acid were effective in protecting against injury by 2% urea spray on kidney bean plants (*Phaseolus vulgarus*). In addition, 10% sucrose sprays prevented a reduction in root dry weight as a result of gibberellic applications (Alvim, 1957).

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, by 0.5 and 1% sucrose. In both cases, 0.5% sucrose effected 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-C^{14} or glucose-C^{14} out of the treated leaf was very slow. Only 1% of the C^{14} from sucrose was translocated after 14 hours. In the dark, 10% of the C^{14} from glucose was translocated to all parts of the seedling in 3 hours. The bulk of the translocated 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 between the effects of sugar

applications in the light versus dark support reason for studying the effects of sugar applications to turfgrass under reduced light conditions.

While most of the exogenous sugar 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.

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 with exogenous sugar applications to plants, an investigation was warranted to determine if exogenous sugar applications can counter the effects of reduced light conditions (shade) for turfgrass situations. In this experiment our objective was to determine whether a physiological response occurs with exogenous fructose applications to turfgrass grown under reduced light conditions. Fructose application frequencies and rates were investigated to determine the positive and/or negative physiological responses when applied to turfgrass under reduced light conditions.

MATERIALS AND METHODS

On 20 September 1999 portable plots were filled with a sand root zone and sodded with one year old supina bluegrass sod (*Poa supina* Schrad.; Manderley Sod Inc., Napean, ON, Canada) using six 1.2 x 1.2 m high-density polyethylene ITM modules (GreenTech, Inc., Richmond, VA, USA) at the Hancock Turfgrass Research Center (HTRC) at Michigan State University, East Lansing, MI, USA. Beginning in May 2000 – January 2001, the plots were fertilized two times per month at 0.5-g N m^{-2} $application^{-1}$ using Lebanon Country Club 18-3-18 fertilizer (Lebanon Seaboard Corp., Lebanon, PA, USA).

On 15 September 2000 the established ITM modules were moved inside the indoor research facility at the HTRC. 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 (Figure 5).

Two studies investigating the effects of exogenous fructose (ISOCLEAR 55, Cargill, Inc., Dayton, OH, USA) applications to supina bluegrass under reduced light conditions were conducted on 4 December 2000 through 26 January 2001 and 4 December 2000 through 12 January 2001 for studies one and two, respectively. The experimental design for these studies was a completely randomized block design with three replications. Statistical analysis was done using Agriculture Research Manager, version 6.18 (Gylling Data Management, Inc. Brookings, SD, USA).

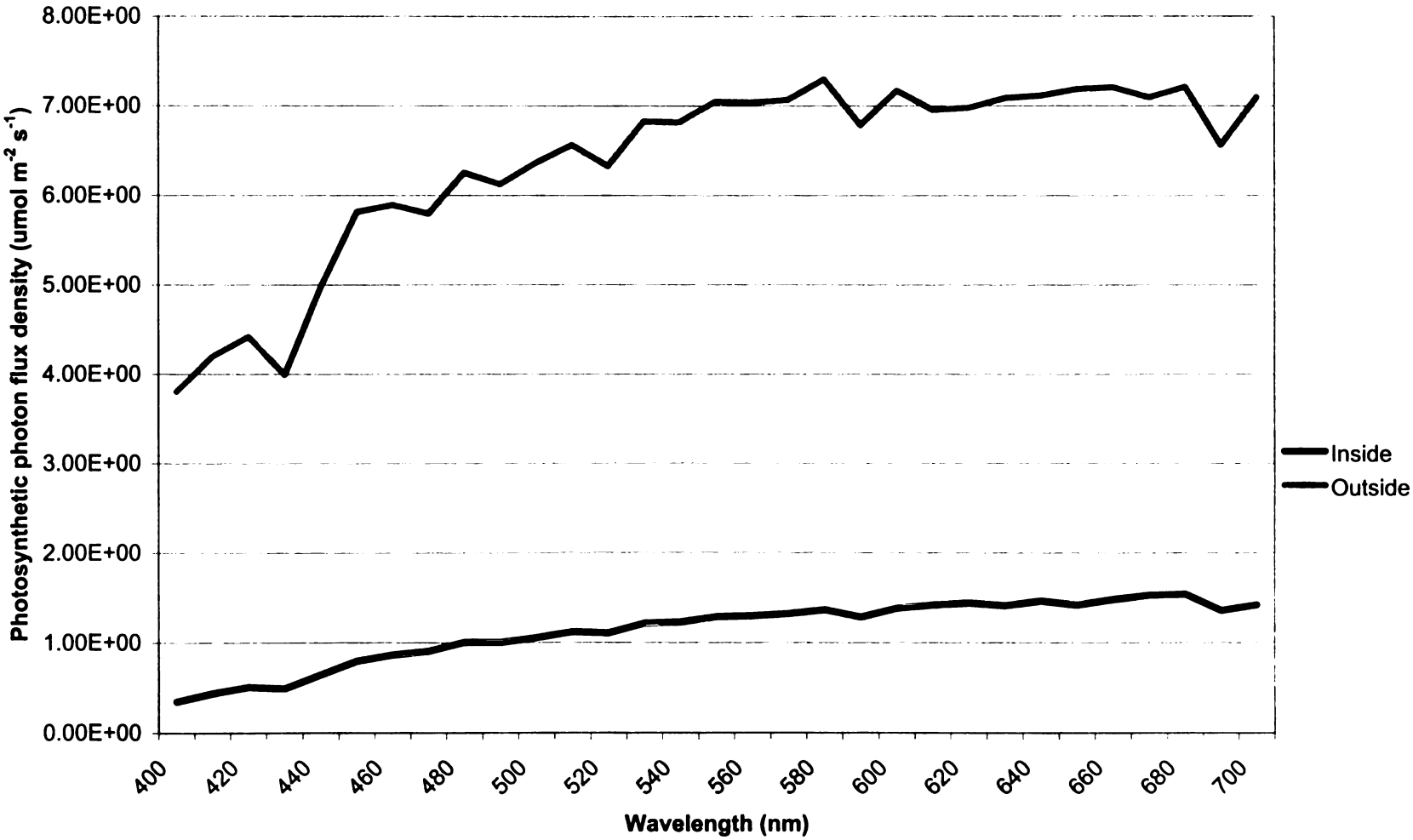

Figure 5. Photosynthetic photon flux density of sunlight and ambient light inside the indoor research facility, East Lansing, MI. 48824. 1430 h, 19 June 2000.

Study one investigated the effects of fructose at 1.25% weight per volume and two different adjuvants (Break Thru® – organosilicone, Aquatrols Inc. Cherry Hill, NJ, USA; Apsa 80® – Alkylaryl alkoxylate, Amway Corp., Ada, MI, USA) at both 0.1 and 0.25% weight per volume applied five times per week for eight weeks. Study two compared the aforementioned treatments using weekly applications for six weeks. Four stock solutions containing the different adjuvant treatments (Break Thru® and Apsa 80® at both 0.1 and 0.25% weight per volume) were made with reverse osmosis water and 1.25% weight per volume fructose. The solutions were applied at 1, 2, 4, 6 and 8 times the rate of 815 L ha^{-1}. Treatments were applied to the leaves as a spray using a 500-ml hand trigger plastic spray bottle. Both of the studies used individual ITM modules as a replication. Plot sizes for treatments were 225 cm^2.

Data collection consisted of visually assessing physiological comparisons of injury and toxicity effects. Turfgrass leaf injury was rated was rated using a 1-9 scale, with 1 being all green with no leaf damage, and 9 being all brown or dead leaf tissue.

RESULTS AND DISCUSSION

Study one *– Five times per week applications*

Significant differences occurred throughout the eight weeks of data collection for both treatments (adjuvants and fructose rate; Table 2). The effects of the two adjuvants (Apsa 80® and Break Thru®) at application rates of 0.1 and 0.25% weight per volume showed significant differences for injury on turfgrass leaf tissue when applied five times per week for eight weeks regardless of the rate of fructose applied (Figure 6). All treatments yielded unacceptable turfgrass leaf tissue quality with the exception of the 0.1% weight per volume rate using the adjuvant Break Thru®.

In addition, different application rates (1, 2, 4, 6, and 8 times 815-L ha^{-1}) of fructose at 1.25% weight per volume, regardless of the adjuvant treatment, showed significant differences for injury to turfgrass leaf tissue when applied five times per week (Figure 7). Only the 1x rate of fructose (815-L ha^{-1}) showed very little injury throughout the duration of the study, suggesting that a rate of 1.25% weight per volume fructose applied at 815-L ha^{-1} is the limit for preventing unacceptable levels of leaf tissue injury (Figure 7).

Table 2. Study one - analysis of variance for leaf tissue injury from exogenous fructose† applications with an adjuvant‡ applied five times per week on supina bluegrass under reduced light conditions. E. Lansing, MI. 48826. 8 December 2000 – 28 January 2001.

		Mean Square Error					
SOURCE	DF	8 Dec.	15 Dec.	30 Dec.	7 Jan.	12 Jan.	28 Jan.
Total	59						
R	2	2.22	0.60	0.02	3.47	3.22	0.00
Adjuvant (A)	3	9.00*	7.26*	6.95*	21.29*	29.71*	71.48*
Fructose rate (B)	4	21.9*	13.61*	9.98*	17.10*	20.11*	35.68*
AB	12	0.82	0.55	0.84	0.49	0.59	2.65*
ERROR	38	0.66	0.86	1.40	0.36	0.71	0.12

† The two adjuvants tested were Break Thru® – organosilicone, Aquatrols Inc. Cherry Hill, NJ, USA; Apsa 80® – Alkylaryl alkoxylate, Amway Corp., Ada, MI, USA) at both 0.1 and 0.25% weight per volume

‡ Fructose application rates were 1, 2, 4, 6, and 8 times 815 L ha^{-1} using 1.25% weight per volume fructose - ISOCLEAR 55, Cargill, Inc., Dayton, OH, USA.

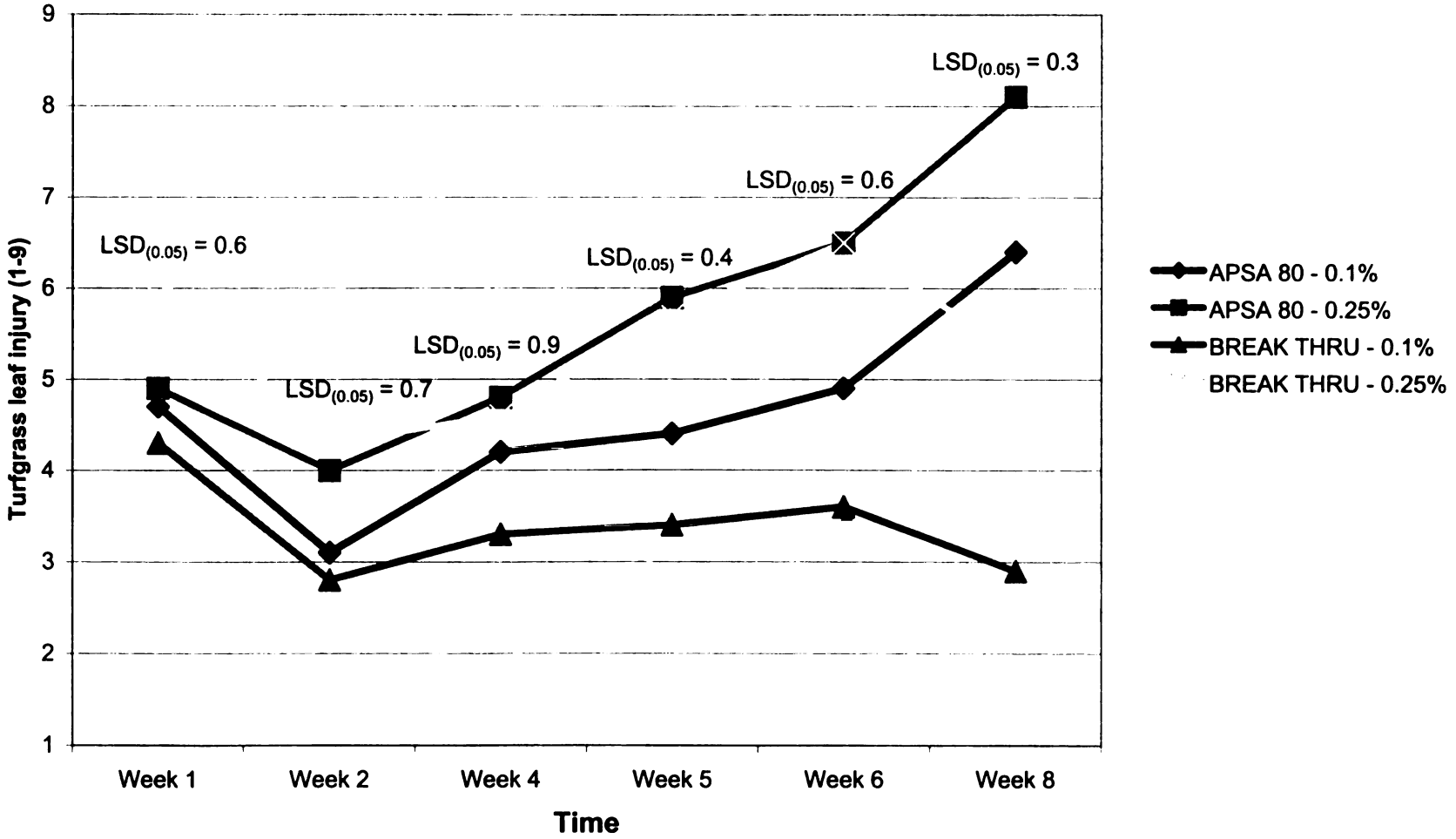

Figure 6. Effects of different adjuvant concentrations for two adjuvants (Apsa 80 - alkylaryl alkoxylate and Break Thru - organosilicone) when applied five times per week with 1.25% weight per volume fructose to supina bluegrass under reduced light conditions. East Lansing, MI. 48824. 8 December 2000 - 27 January 2001.

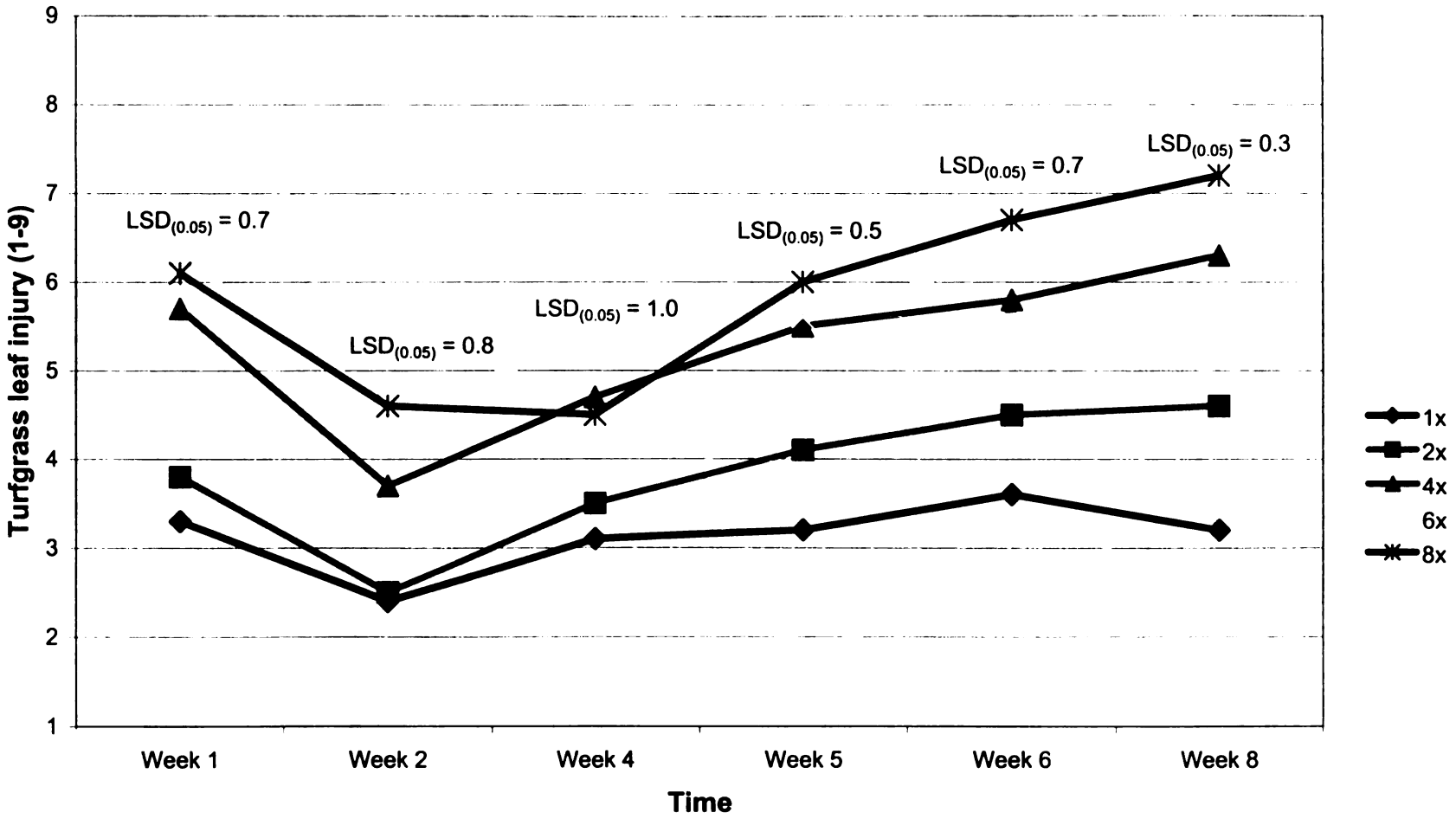

Figure 7. Effects of 1, 2, 4, 6, and 8 times the 815 L ha^{-1} application rate for 1.25% weight per volume fructose applied to supina bluegrass under reduced light conditions five times per week. East Lansing, MI. 48824. 8 December 2000 - 27 January 2001.

Study two *– One time per week applications*

After six weeks of testing, significant differences in turfgrass leaf tissue injury occurred between weekly applications with the two adjuvants (Table 3). Regardless of the adjuvant, the rate of 0.1% weight per volume adjuvant in solution caused significantly less tissue injury than the rate of 0.25% weight per volume in solution (Figure 8).

Significant differences in turfgrass leaf tissue injury was observed with different application rates of fructose (Table 3). The 1x rate of fructose (815-L ha^{-1}) showed very little injury throughout the duration of the study, suggesting that a rate of 1.25% weight per volume fructose applied at 815-L ha^{-1} is ideal for preventing leaf tissue injury (Figure 9). In addition, 1.25% weight per volume fructose applied at 2, 4, 6, and 8 times the 815-L ha^{-1} yielded acceptable turfgrass leaf injury levels. These results suggest that weekly (one time per week) exogenous fructose applications are more practical than daily (five times per week) applications for turfgrass under reduced light conditions.

For supina bluegrass growing under reduced light conditions the rate of 1.25% weight per volume fructose applied at 1, 2, 4, 6, and 8 times 815-L ha^{-1} is acceptable in terms of injury and triggers positive physiological changes to occur compared to the control plants where no fructose applications were applied (Figure 9). These observational comparisons suggest that the plant is readily absorbing exogenous fructose applications and is using it for metabolic processes.

Table 3. Study two - analysis of variance for leaf tissue injury from exogenous fructose† applications with an adjuvant‡ applied one time per week on supina bluegrass under reduced light conditions. E. Lansing, MI. 48826. 8 December 2000 – 12 January 2001.

		Mean Square Error				
SOURCE	DF	8 Dec.	15 Dec.	30 Dec.	7 Jan.	12 Jan.
Total	59					
R	2	6.47	0.17	0.47	0.35	0.72
Adjuvant (A)	3	3.78*	1.31	1.00	1.35*	2.55*
Fructose rate (B)	4	10.54*	1.56	1.81*	1.43*	2.60*
AB	12	0.88	1.05	0.43	0.27	0.30
ERROR	38	1.06	0.68	0.38	0.32	0.42

† The two adjuvants tested were Break Thru® – organosilicone, Aquatrols Inc. Cherry Hill, NJ, USA; Apsa 80® – Alkylaryl alkoxylate, Amway Corp., Ada, MI, USA) at both 0.1 and 0.25% weight per volume

‡ Fructose application rates were 1, 2, 4, 6, and 8 times 815 L ha^{-1} using 1.25% weight per volume fructose - ISOCLEAR 55, Cargill, Inc., Dayton, OH, USA.

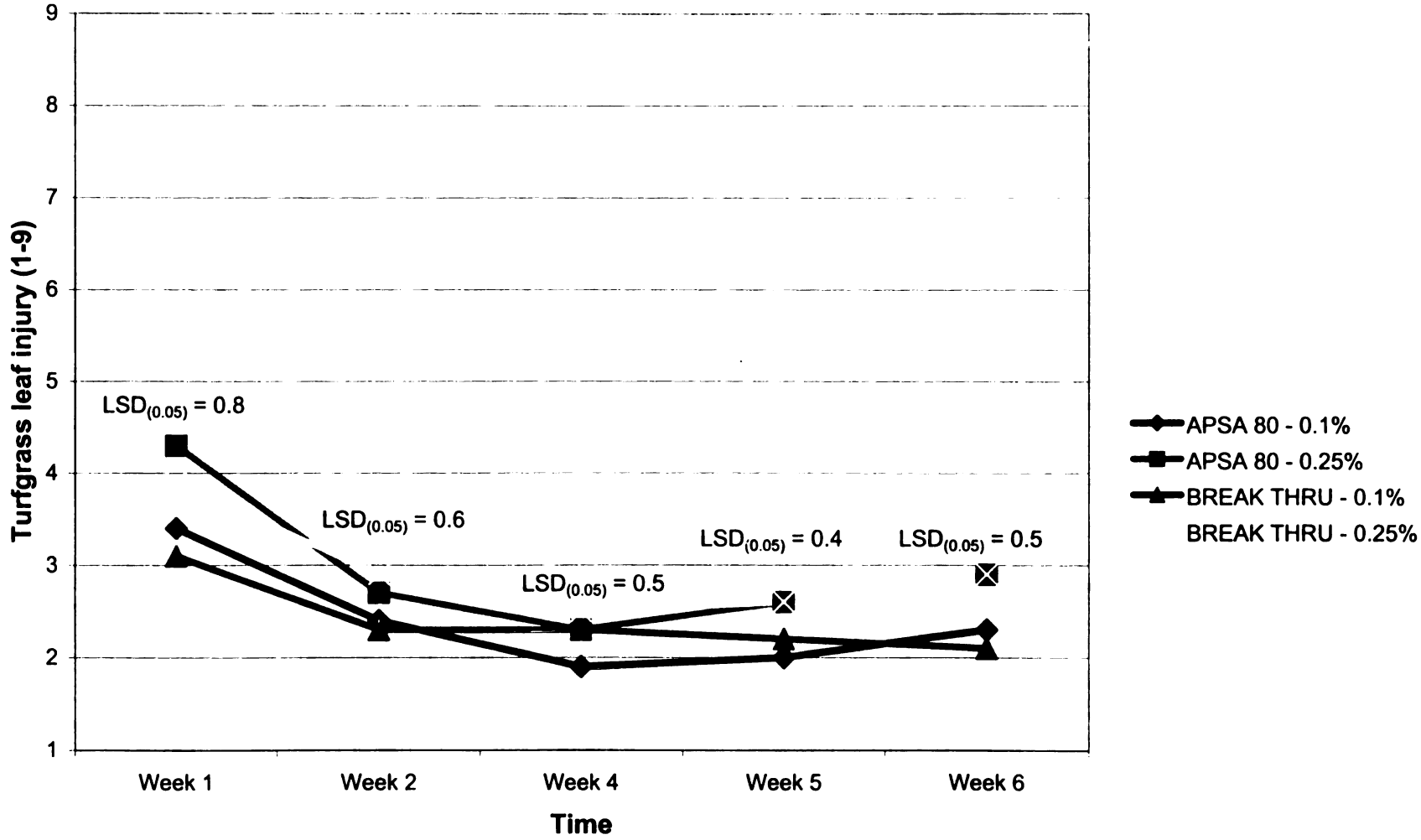

Figure 8. Effects of different adjuvant concentrations for two adjuvants (Apsa 80 - alkylaryl alkoxylate and Break Thru - organosilicone) when applied once per week with 1.25% weight per volume fructose to supina bluegrass under reduced light conditions. East Lansing, MI. 48824. 8 December 2000 - 27 January 2001.

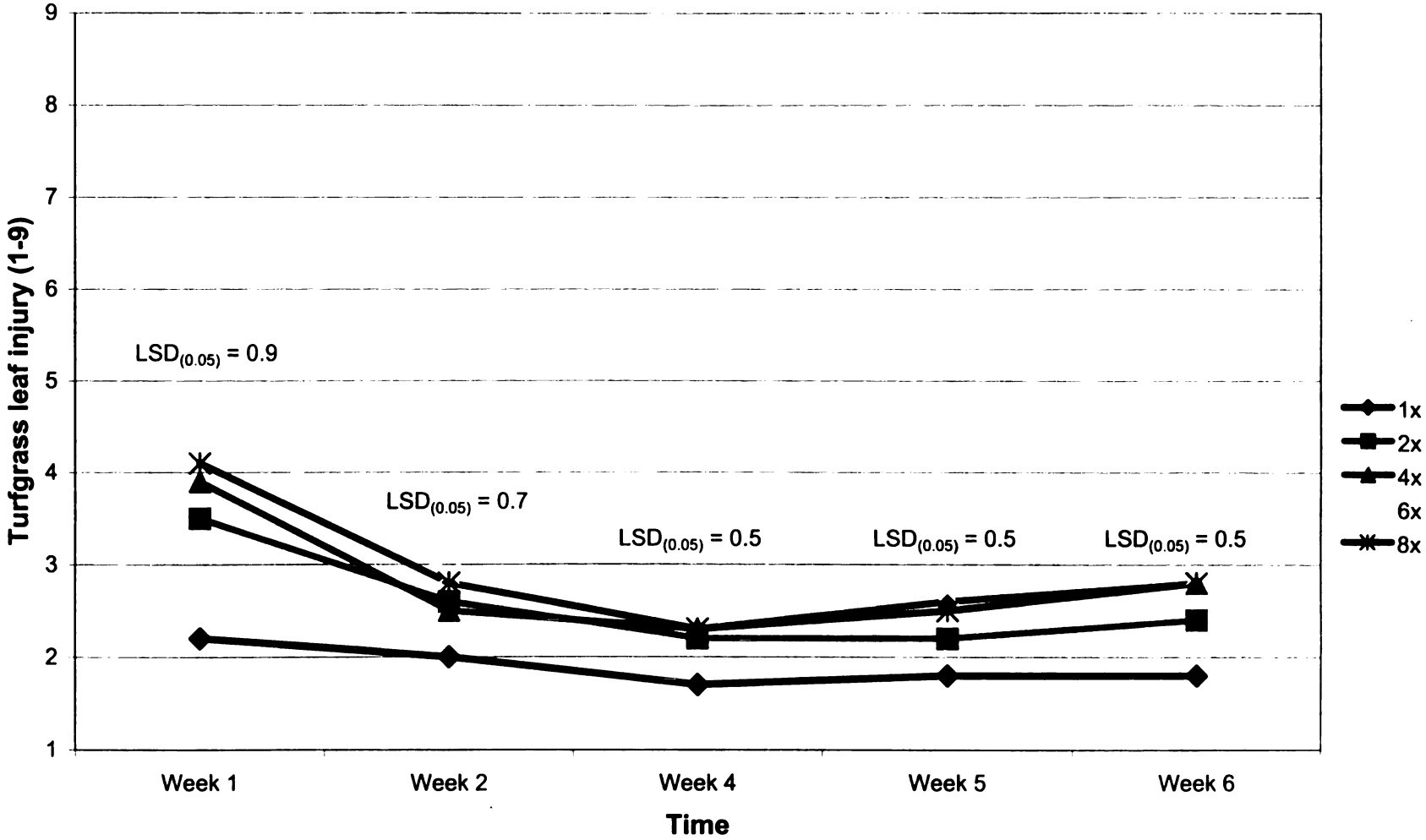

Figure 9. Effects of 1, 2, 4, 6, and 8 times the 815 L ha^{-1} application rate for 1.25% weight per volume fructose applied to supina bluegrass under reduced light conditions once per week. East Lansing, MI. 48824. 8 December 2000 - 27 January 2001.

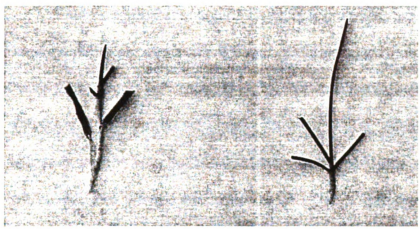

Figure 10. Digital illustration demonstrating physiological differences between two supina bluegrass plants grown in reduced light conditions. The plant on the left has received weekly exogenous fructose applications for six weeks and the plant on the right has not received any fructose, E. Lansing, MI 12 Jan. 2001

CONCLUSIONS

Fructose applied to supina bluegrass under reduced light conditions five times per week for eight consecutive weeks causes too much leaf injury unless the application rate is 1.25% weight per volume fructose at the 815-L ha^{-1} rate with 0.1% weight per volume Break Thru® adjuvant. Weekly fructose applications (one time per week) yielded acceptable levels of leaf tissue injury for the 1,2,4,6, and 8 times application rates. The 1x rate provided the least amount of leaf tissue injury while demonstrating positive physiological responses compared to the control. Overall, this study was successful as a preliminary study to warrant more in-depth research for understanding the uptake and translocation of exogenous fructose applications to turfgrass under reduced light conditions.

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Chapter 3

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

ABSTRACT

Turves 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}C6 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 vulgarus*) 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-C^{14} or glucose-C^{14} out of the treated leaf was very slow. Only 1% of the C^{14} from sucrose was translocated after 14 hours. In the dark, 10% of the C^{14} from glucose was translocated to all parts of the seedling in 3 hours. The bulk of the translocated 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.

MATERIALS AND METHODS

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.

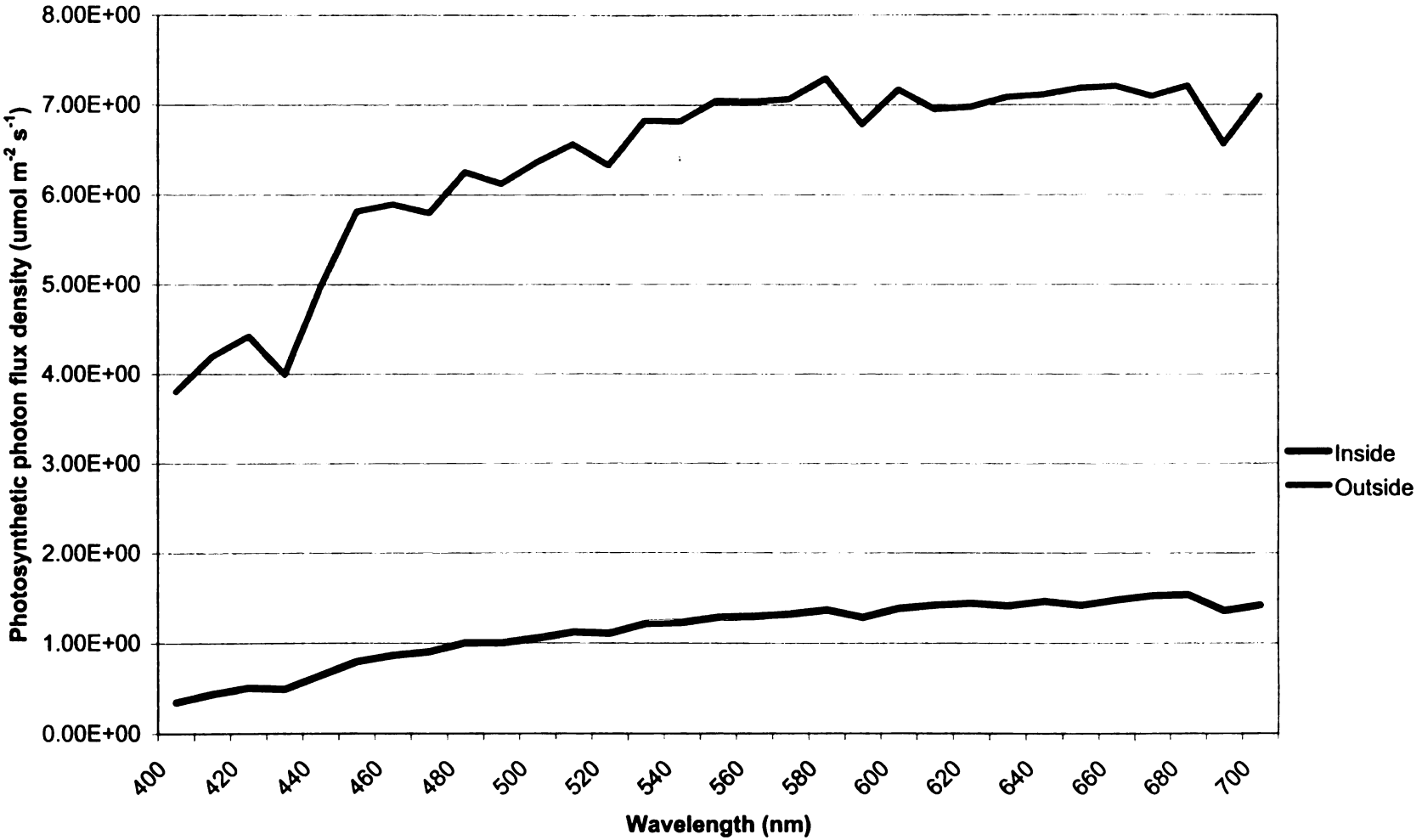

Figure 11. Photosynthetic photon flux density of sunlight and ambient light inside the indoor research facility (20% +/- 3% ransmittance), East Lansing, MI. 48824. 1430 h, 19 June 2000.

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}C enrichment. Stable C isotope ratios were determined and correction factors applied according to Craig (1957), and the results are expressed as

Eq. 1.
$$\delta^{13}C_{PDB} = \left[\frac{R_{sample}}{R_{standard}} - 1 \right] x\ 10^3$$

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

In addition to the $\delta^{13}C_{PDB}$ value, the absolute ratio (R), fractional abundance (F), and atom % were other indices used to determine stable isotope abundance from the ^{13}C enrichment. Absolute ratio, fractional abundance and atom % rearrange $\delta^{13}C_{PDB}$ in order to calculate the amount of ^{13}C in plant tissues (Boutton, 1991, and Svejcar, *et al.*, 1985).

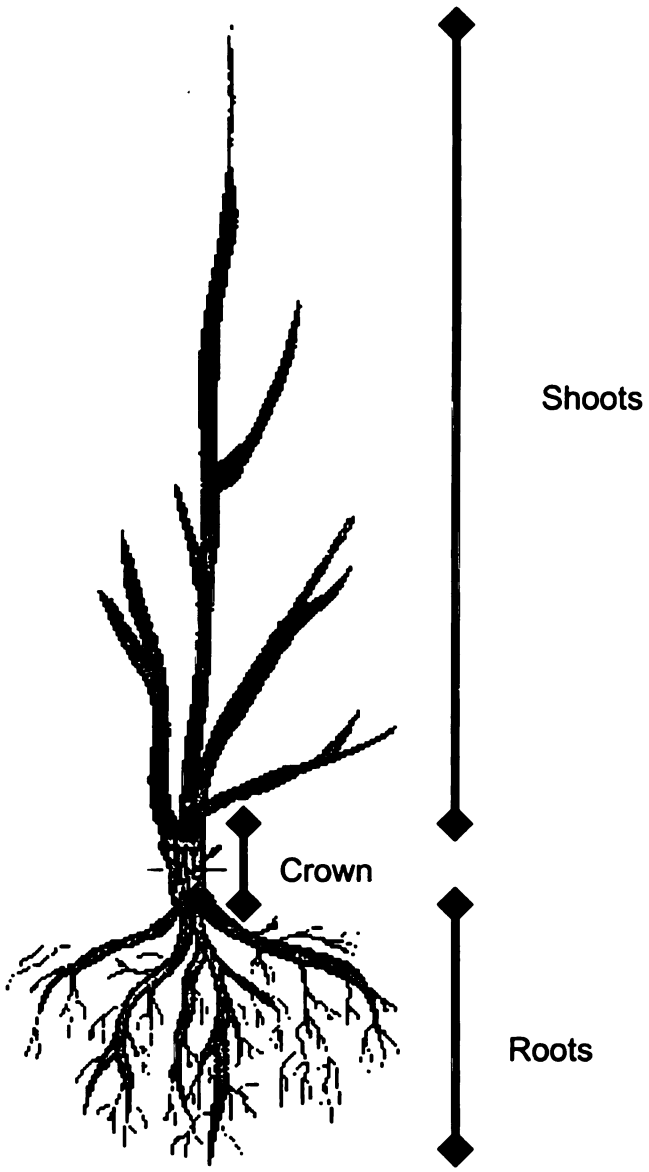

Figure 12. Illustration of turfgrass plant tissue shoot - crown - root separation.

The absolute ratio of a sample is defined by rearrangement of Eq. 1 as:

Eq. 2. $$R_{sample} = {}^{13}C/{}^{12}C = \left[\frac{\delta^{13}C}{1000} + 1\right] \times R_{PDB}$$

The fractional abundance is related to R by the equation:

Eq. 3. $$F = \frac{{}^{13}C}{{}^{13}C + {}^{12}C} = \frac{R}{R + 1}$$

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

Eq. 4. Atom % = F x 100

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 % ($^{13}C/^{12}C$) 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 % ($^{13}C/^{12}C$) was greatest for both one and five fructose applications in the shoots suggesting the shoots as the primary plant tissue for ^{13}C structural synthesis. One and five applications of fructose also increased the atom % ($^{13}C/^{12}C$) 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.

SOURCE	DF	MSE
Total	26	
R	2	0.01
Tissue† (A)	2	0.44*
Time‡ (B)	2	0.60*
AB	4	0.17*
ERROR	16	0.03

* 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.

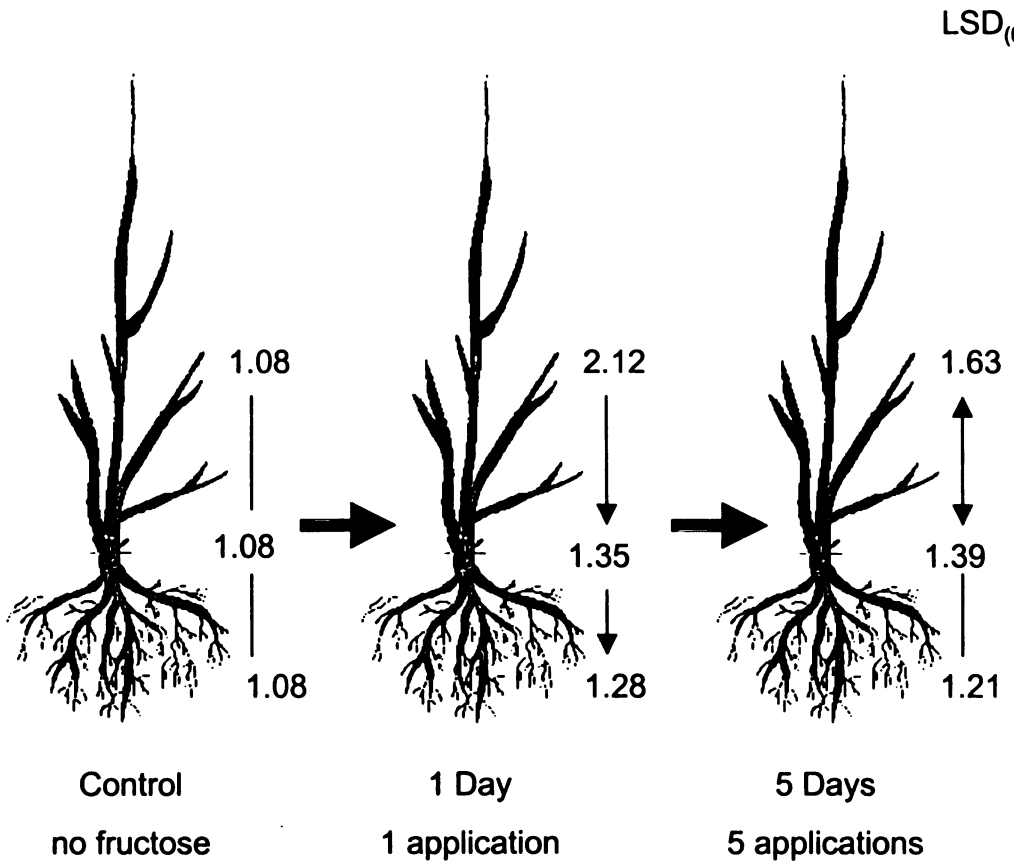

Figure 13. Plant tissue (shoots:crown:roots) over time atom % ($^{13}C/^{12}C$) interaction for consecutive ^{13}C-fructose applications to supina bluegrass under reduced light conditions. East Lansing, MI. 48824. 10 - 19 September 2001.

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 % ($^{13}C/^{12}C$) from exogenous ^{13}C-fructose applications to supina bluegrass under reduced light conditions. E. Lansing, MI. 48824. 10 – 27 Sep. 2001.

SOURCE	DF	MSE
Total	35	
R	2	0.19
Tissue† (A)	2	0.14
Time‡ (B)	3	0.27*
AB	6	0.07
ERROR	22	0.06

* Significant to the 0.05 probability level.

† Tissue consists of shoot – crown – root separation.

‡ Time consisted of sampling a control treatment, and samples collected 1, 8, and 13 days after five days of exogenous fructose applications.

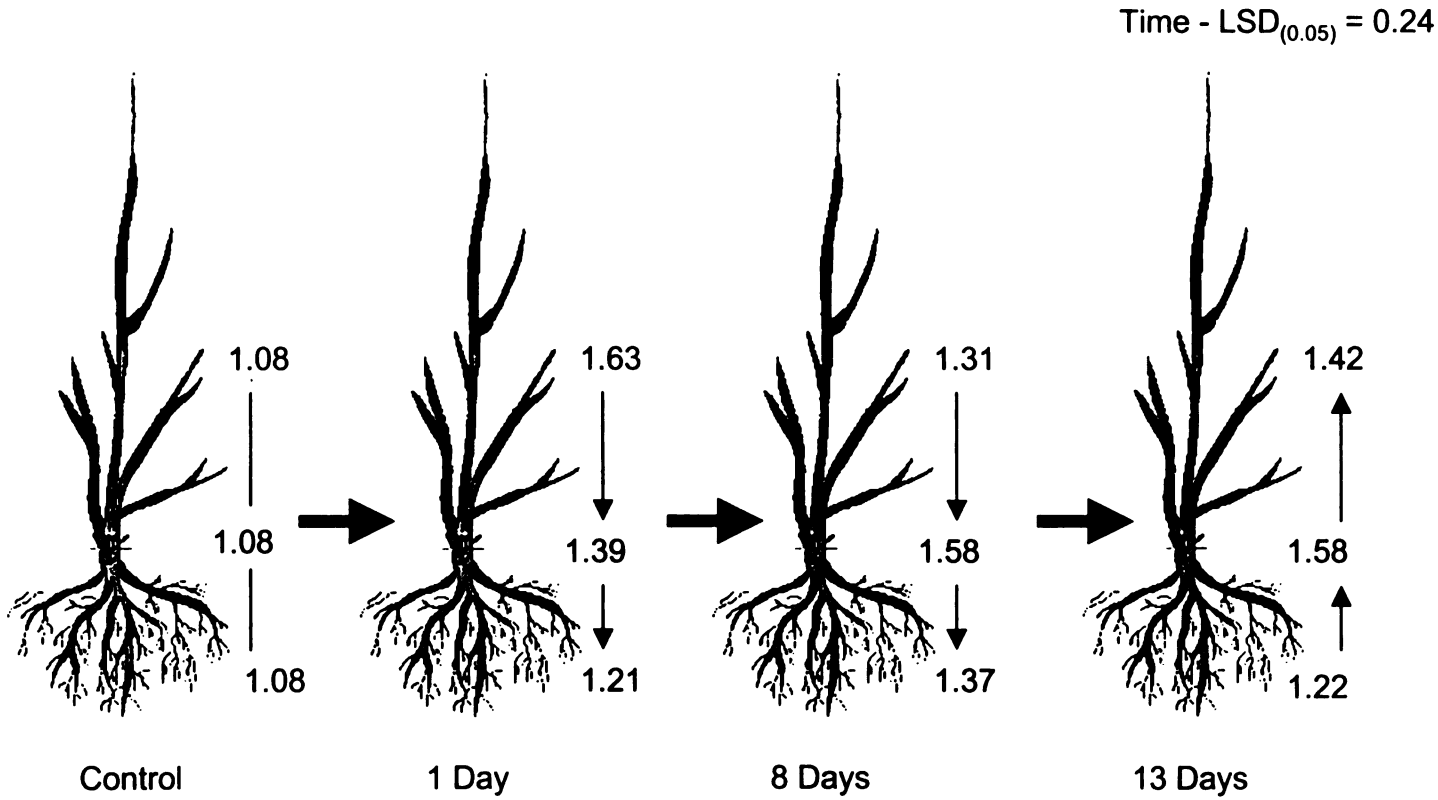

Figure 14. Atom % ($^{13}C/^{12}C$) significance over time with source sink relationship on supina bluegrass plant tissue (shoots:crown:roots) after five days of exogenous ^{13}C-fructose applications. East Lansing, MI. 48824. 10-27 September 2001.

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.

SOURCE	DF	MSE
Total	44	
R	2	0.15
Tissue† (A)	2	0.58*
Time‡ (B)	4	2.64*
AB	8	0.13*
ERROR	28	0.03

* 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.

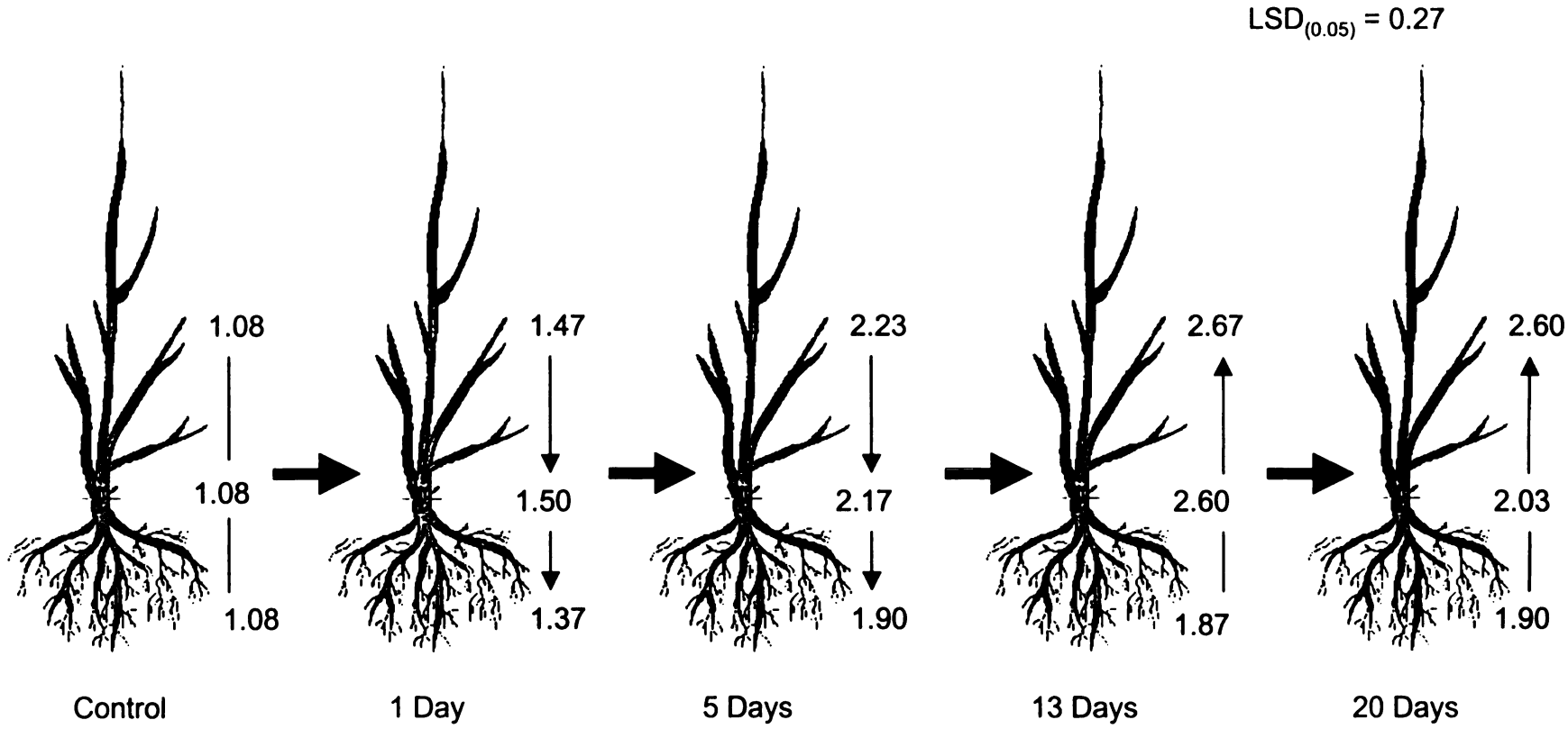

Figure 15. Plant tissue (shoots:crown:roots) over time atom % ($^{13}C/^{12}C$) interaction on supina bluegrass under reduced light conditions after one exogenous ^{13}C-fructose application. East Lansing, MI. 48824. 30 November - 21 December 2001.

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.

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DISSERTATION SUMMARY

Discoveries from the previous three chapters will provide valuable insight for the future management of turfgrass under reduced light conditions (shade). The positive turfgrass response to exogenous fructose applications introduces an economical and environmentally safe method for managing turfgrass in the shade. Following are conclusions for each of the three chapters.

***Chapter one* – Determination of shade tolerance for three cool-season turfgrass species (Kentucky bluegrass, tufted hairgrass, and tall fescue) using $^{13}CO_2$ pulse chase procedures.**

The $^{13}CO_2$ pulse chase experiments were done on turfgrasses where no other foreign stresses (traffic, nutrient deficiencies, water stress, etc.) where present. Results for this experiment determined that the reduced light levels provided by the indoor research facility are not limiting enough for tall fescue and tufted hairgrass, thus suggesting their effective shade tolerance. Because tall fescue and tufted hairgrass are slow growing turfgrasses, the level of reduced light did not limit their photosynthetic requirements. Conversely, the $^{13}CO_2$ pulsing suggested that the shade tolerance of Kentucky bluegrass, a more aggressive growing turfgrass, to be insufficient under the reduced light conditions. Therefore, successful determination of turfgrass shade tolerance for each species with respect to the reduced light conditions from the indoor research facility was determined using $^{13}CO_2$ pulse chase experiments.

Chapter two* – *Effects of exogenous fructose and adjuvant applications on leaf injury of supina bluegrass under reduced light conditions.

Fructose applied to supina bluegrass under reduced light conditions five times per week for eight consecutive weeks causes too much leaf injury unless the application rate is 1.25% weight per volume fructose at the 815-L ha^{-1} rate with 0.1% weight per volume Break Thru® adjuvant. Weekly fructose applications (one time per week) yielded acceptable levels of leaf tissue injury for the 1,2,4,6, and 8 times application rates. The 1x rate provided the least amount of leaf tissue injury while demonstrating positive physiological responses compared to the control. Overall, this study was successful as a preliminary study to warrant more in-depth research for understanding the uptake and translocation of exogenous fructose applications to turfgrass under reduced light conditions.

Chapter three* – *Determination of the absorption and translocation of exogenous ^{13}C-fructose applications to supina bluegrass under reduced light conditions.

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.

What next?

The discoveries ascertained during these experiments yielded useful information for the management of turfgrass under reduced light conditions. However, like a lot of research projects, the question of what next is very pronounced. While, it has been determined that weekly fructose applications are better than applications applied five times per week, further research is warranted. Specific understanding of the optimal application rate and frequency for different turfgrass species under reduced light conditions in necessary.

The long term effects of continuous fructose applications to a turfgrass stand under reduced light conditions needs to be studied. Specifically the effects on plant biochemical processes like enzymatic functions, carbohydrate synthesis and storage, etc. In addition, the effects of continuous fructose applications on soil properties, microbial activity, weeds, insects, fungi, etc. need to be understood.

One component that needs to be determined is what level of photosynthetic carbohydrate synthesis is required. Simply applying exogenous fructose applications to turfgrass under different light levels, including no light, will define the limits of how much shade it too much; particularly, when the

indirect stimuli from photosynthesis is restricted. In addition, the effects exogenous fructose applications have for plant photosynthesis needs to be investigated.

The discoveries from the previous three chapters will provide valuable insight for the future management of turfgrass under reduced light conditions as well as open up a plethora of research opportunities for continued understanding and discoveries. The future looks **SWEET!!!**

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