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The Effect Of Exogenous Fructose On Creeping Bentgrass Heat Tolerance

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THE EFFECT OF EXOGENOUS FRUCTOSE ON CREEPING BENTGRASS HEAT TOLERANCE

By

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Creeping bentgrass (*Agrostis stolonifera)* used on golf course putting greens are some of the most intensively managed areas of turf and are subjected to high stress. Heat stress results in lowered photosynthetic efficiency and inadequate sugar production. An exogenous application of fructose could compensate for the lack of sugar being produced. The objectives of this research were to determine the effect of exogenous applications of fructose on heat stressed creeping bentgrass. Field results showed some phytotoxicity with high rates of fructose, while lower rates showed no visible damage compared to an untreated control. Low rates of surfactant resulted in little phytotoxicity, while high surfactant rates showed damage. Fructose had no positive effect on turf quality. A surfactant study was then designed to measure the effect of various surfactants on fructose uptake. This study revealed that as hydrophilic to lipophilic balance increased, absorption of fructose increased.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Turfgrass is an integral part of the ecosystem and the world economy. It has been estimated that around 50 million acres of turfgrass is maintained for some type of use within the United States with an economic value of $40 billion (Beard et al., 2006). Turfgrass has been shown to improve quality of life by increasing property value, conserving natural resources, and providing open space and recreational opportunities (Fender et al., 2008). Turfgrass provides many career opportunities such as grounds superintendent, manufacturing/sales representative, professional-service contractor, technical writer, and scientist/educator (Turgeon, 2008).

Creeping bentgrass (CBG) (*Agrostis stolonifera* L.) has been used on everything in turf management from home lawns to golf courses. However, its dense canopy, fine texture, spreading stolons, and low mowing tolerance, make it the most popular and highest quality choice for golf course fairways and putting greens (Christians, 1998; Beard 2002; Koh et al., 2003; Turgeon, 2008; Cooper and Peacock 2008). The popularity and publicity of golf has associated CBG putting greens with premier facilities. This demand has resulted in it being spread to areas not adapted for its optimal growth (Christians, 1998). The use of this grass in the south was also a result of a lack of high putting quality warm-season options for golf greens. Bentgrasses were heavily developed by the USGA in the mid 1900s and culminated with the release of 'Penncross' in 1955 (Turgeon, 2008). However, breeding of short-statured bermudagrasses for warmer environments did not begin until 10 years later (Turgeon, 2008). While CBG does present an excellent putting surface, the amount of labor, chemicals, and irrigation required to keep this surface alive south of the transition zone is insufficient during some years (Dernoeden, 2002).

When grown in areas where CBG is not adapted, the turf must receive constant care during the summer months. On golf course putting greens, extensive measures are taken to keep the turf alive. Since most greens are sand based, the water holding capacity is minimal (Beard, 2002). As a result, the greens must be watched on hot afternoons and syringed as needed to prevent desiccation and pest injury (Waddington et al., 1992). Syringing is the process of applying just enough water to wet the leaves without wetting the soil underneath. As the canopy moisture evaporates, a cooling effect results (Dernoeden, 2002). Beard (1973) found a reduction of 2.22°C in turf canopy temperatures and a 1.67°C in soil temperatures after applying 0.635 cm of water at noon to CBG located in East Lansing, MI. Lowering midday temperatures also helps the turf to reach more optimal, cooler, night time temperatures more quickly so it has longer to recuperate (Guertal et al., 2005).

Although these mid-day irrigation events can be helpful, too much water is also unhealthy for plants. Excess water can lead to excellent conditions for fungi to colonize turf that is already stressed from the heat (Smiley et al., 2005). As another precaution against fungal growth, CBG greens are commonly sprayed once a week with a mixture of fungicides and low analysis fertilizers to suppress or prevent fungal growth and encourage growth of the turf. As a way to get water in the root zone and relieve soil compaction, high-pressure water injection or needle tines are used by some superintendents on a regular basis. Wetting agents are another method of getting water to the rootzone and reducing hydophobicity. Industrial size fans along the border of the greens are sometimes utilized to keep air circulating. Air movement helps cool the heat stressed leaves by reducing turf mat and soil temperatures (Guertal et al., 2005). Further, these fans also disturb the boundary layer surrounding the turf leaves. The boundary layer is a layer of moisture that forms as water transpires from the plant and proportional to the transpiration rate, relative humidity, and wind velocity (Turgeon 2008). If the boundary layer is disturbed, then the vapor pressure gradient between the leaf and the air is reduced, thus increasing transpiration rates (Taiz and Zeiger, 1998; Turgeon 2008). All these management techniques also require large amounts of man hours on the putting green, which disrupts play of the course. Management strategies such as these aimed at keeping this putting surface alive results in massive uses of electrical, water, chemical, and labor resources and still may not be able to prevent a decline in turf quality which translates to a poor putting surface (Guertal et al., 2005).

As a result of adverse climate conditions, CBG begins to show signs of stress. At high temperatures, root and leaf function begin to decline. During the optimal growing months in the spring or autumn, roots can extend 10 to 20 cm into the soil (Sprague, 1933). When soil temperatures rise above 27^{o}C, root growth begins to slow and eventually stops (Carrow, 1996). Unable to function at full capacity, roots begin to slough off and can recede to lengths of 1.25 to 5 cm. Shortened roots during the summer decrease the nutrient uptake zone and may magnify the already poor growing ability, resulting in decreased water and nutrient uptake. (Dernoeden, 2002).

To counteract reduced water and nutrient uptake by damaged roots, greens must be watered more frequently to provide water and reduce canopy temperatures. As was previously mentioned, this is accomplished by syringing and deep, infrequent irrigation. Water must be applied by these methods so as to create the least amount of disease pressure as possible. Prolonged leaf wetness is a major factor in disease activity (Dernoeden, 2002). Heat stressed CBG subjected to periods with no moisture will initially turn a brownish color and then begin to die (Guertal et al., 2005). With no new growth, the turf will begin to thin, making playing conditions and visual appearance unsuitable. However, overwatered turf is as much of a problem as insufficient water. Due to the high specific heat of water, during especially hot days, excess water can heat to temperatures warmer than ambient temperatures. Scalding of turf can occur in saturated areas from abundant watering. Thus, moisture-trapping areas such as thatchy or low lying areas can ultimately result in plant death (Turgeon, 2008). Wet wilt is another water related issue that occurs when the plant is transpiring faster than the roots can absorb water (Turgeon, 2008). When the soil is saturated, all the pore space is occupied, replacing all of the oxygen found in the soil with water leaving the roots unable to respire (Brady, 2002).

Stressed turf is very susceptible to fungal growth. Brown patch (*Rhizoctonia solani*), dollar spot (*Sclerotinia homoeocarpa*), anthracnose (*Colletotrichum* *graminicola*), and pythium (*Pythium* ssp.) are a few of the common summer diseases experienced on CBG putting greens (Dernoeden, 2002). Diseases are often hard to diagnose, and the time and materials used to correct these problems can be costly. Plant parasitic nematodes are very prevalent on sandy soils and can cause major damage to turf root systems. Nematode feeding and fungal activity can increase during high temperatures leading to enhanced damage to roots already struggling from heat stress (Dernoeden, 2002).

These heat stress problems may at least partially be due to CBG' photosynthetic pathway. Photosynthesis is a process where plants use CO_2, water, and the sun's light for energy to produce compounds needed for their growth (Christians, 1998). Photosynthesis is utilized by all green plants and is commonly represented by the formula:

$$6CO_2 + 6H_2O \rightarrow C_6H_{12}O_6 + 6O_2$$

This photosynthetic reaction occurs in the chloroplasts of plant cells. When in the presence of light, the water molecules are split into H^+ and O_2^- in the thylakoid membranes within the chloroplast. The free electrons generated from O_2 during the light reaction are used to reduce CO_2 to simple sugars.

When CBG is subjected to extreme stress, it is unable to carry out photosynthesis to full potential, leading to the turf quality reducing factors mentioned above. When C_3 plants are subjected to high temperatures, the plant begins a process known as photorespiration. This efficiency-robbing pathway binds O_2 rather than CO_2 at high temperatures, allowing only one molecule of 3-phosphoglyceric acid (3 PGA), the precursor to glucose, to be formed rather than two. As a result, no sugar can be generated and CO_2 is released back into the atmosphere (Turgeon, 2008). Without the main sugar being produced from photosynthesis, respiration levels decrease resulting in slower leaf and root growth from lack of energy.

As the plant is conducting photosynthesis, the production of carbohydrates usually exceeds the use of carbohydrates (Waddington et al., 1992). Cool-season grasses tend to store carbohydrates as long chain polymers of fructose molecules, or fructans (Waddington et al., 1992). However, due to photorespiration, plants do not make sugars and thus have reduced energy stores of fructans to utilize when growing conditions become unfavorable and the plant is unable to conduct photosynthesis.

In order to keep CBG greens at satisfactory conditions during stressful periods, managers will employ every possible stress-relieving tactic. Unless there is an alternative energy supply available to the plant, the use of the previously mentioned management practices will, at best only help to keep the plant functioning at a much reduced state. The combination of all these methods can be very expensive and still not offset the problem of photorespiration. Rather than trying to combat the effects of stress from photorespiration, applying a source of sugar may counteract the problem of photorespiration. However, very little research exists in the literature on providing plants with supplemental sugars during stressful periods. Juhren and Went (1949) applied sucrose to squash (*Cucurbita pepo* var. 'Table Queen') plants grown in darkness and found an increase in lifespan. Berrie (1959) conducted a study on applying sucrose spray to tomato (*Lycopersicum* esculentum Mill.) plants grown at varying light and temperature levels, and found an increase in dry weight and development. Sorochan (2002) applied fructose to *Poa supina* Schrad. grown under reduced light conditions and saw positive physiological response. No known research has explored applying sugar to heat stressed plants. However, it is well established that carbohydrate levels suffer during summer months due to limited sugar production (Smith, 1968; Solhaug, 1991).

Photosynthetic Pathways

Creeping bentgrass' photosynthetic pathway provides evidence as to why this species often struggles during hot weather. Photosynthesis involves a pathway where CO_2, H_2O, and the sun's energy are necessary to eventually produce substances needed for growth (Christians, 1998). It is utilized by all green plants and is commonly represented by the formula:

$$6CO_2 + 6H_2O \rightarrow C_6H_{12}O_6 + 6O_2$$

Photosynthesis occurs in the chloroplasts of plant cells. The first step of this process involves the uptake of CO_2 and H_2O along with the capture of sunlight. When in the presence of light, the water molecules are split into H^+ and O_2^-. The excited electrons are harvested from these elements and ultimately stored as NADPH, a reducing agent. Energy in the form of ATP is also generated through photophosphorylation (Campbell et al., 2008). All of the happenings in the thylakoid membranes within the chloroplast are referred to as the light reactions. The free electrons generated from O_2 during the light reactions are used to reduce CO_2 to simple sugars in a step referred to as the light independent reactions. As the name implies, light is not required for this step. Similar to the light reactions, this step also occurs in the chloroplast, however, this series of reactions happen outside the thylakoids in the stroma. The process of CO_2 fixation typically varies between two pathways commonly referred to as the Calvin-Benson Cycle (C_3) and the Hatch and Slack (C_4) pathway. Cool-season grasses follow the C_3 pathway where as warm-season grasses follow the C_4 pathway. The difference between the two pathways accounts for the adaptability of different turf species to different geographical areas.

The C_3 pathway was first described by Melvin Calvin and Andrew Benson in the 1950s (Bassham et al., 1950). When CO_2 enters a plant, a portion is hydrolyzed to form carbonic acid (H_2CO_3) to become part of the aqueous medium of the cell (Turgeon, 2008). While in solution, H_2CO_3 is further altered to form a bicarbonate anion (HCO_3^-). Plants following the C_3 pathway are only able to utilize a portion of the CO_2 entering the leaf as their receptor (RuBP) will only accept CO_2 in the gaseous form (Waddington et al., 1992). Within the mesophyll, CO_2 enters the light independent reactions and binds to the 5-carbon sugar, ribulose-1,5-bisphosphate (RuBP). The now six-carbon molecule immediately splits into two molecules of the three carbon compound 3-phosphoglyceric acid (3 PGA) (Turgeon, 2008). One source of energy created from the light reaction, $NADPH_2^+$, is used to reduce the two PGA molecules into two molecules of 3-phosphoglyceraldehyde (PGAL) (Turgeon 2008). The majority of the PGAL continues on to complete the cycle by regenerating RuBP. The part left behind makes up 1/6 of a glucose molecule. Thus, for every 6 turns of the cycle (or every 6 CO_2 molecules entering the cycle), one molecule of glucose is generated.

The C_4 pathway was discovered in the 1960s by two research groups working with sugarcane (Slack and Hatch, 1967). Plants classified as C_4 plants contain an additional pathway to C_3 plants, occurring in both mesophyll and bundle sheath cells (Turgeon, 2008). Further, C_4 plants possess a different receptor molecule for CO_2. Within the mesophyll cell, the receptor molecule, phosphoenolpyruvate (PEP), is able to receive CO_2 in the bicarbonate form in the presence of the enzyme PEP carboxylase (Waddington, 1992). This binding forms oxaloacetic acid, (OAA), a four-carbon compound and the namesake for the C_4 pathway (Turgeon 2008). Oxaloacetic acid is reduced to either malic acid or aspartic acid and then sent to the adjacent bundle sheath cells (Turgeon 2008). At this point the two acids are decarboxylated into pyruvic acid and can be phosphorylated to regenerate PEP in the mesophyll (Turgeon 2008). The remaining CO_2 is sent to the C_3 cycle for continued fixation.

Inefficiency of C_3 Plants

Plants using C_3 photosynthesis have several characteristics that make them less efficient in high temperatures than C_4 plants (Turgeon, 2008). The major problem associated with C_3 plants during high temperatures is their inability to produce enough photosynthate to conduct respiration needed for growth. As mentioned previously, the products from photosynthesis are glucose, oxygen, and water. As a result of photosynthesis, the plant loses water. During the hottest portion of the day, as a defense mechanism, plants are able to close stomates to stop the loss of water. This is necessary to prevent the plant from essentially drying up. However, these stomates are also the openings in which CO_2 enters the plant. The plant is only able to hold certain amounts of CO_2. In C_4 plants, when CO_2 enters the plant and is changed to the bicarbonate form HCO_3^- (Waddington et al., 1992), the plant is able to store this product until it is ready to be bound to PEP. When stomates close, there is a reserve of HCO_3^- in solution from which the plant can draw. In C_3 plants RuBP carboxylase is only able to bind the gaseous form of CO_2 (Waddington et al., 1992). It is not able to store this form in solution. When temperatures rise and the stomates close, C_3 plants have no CO_2 reserve from which to pull. With the stomates closed and the plant still conducting photosynthesis, the concentration of available CO_2 soon becomes depleted.

Another factor contributing to the reduced concentration of CO_2 within the plant deals with the physical and chemical properties of CO_2 and O_2. The solubility of both gases decreases as temperatures rise, which also decreases the concentration of each in the plant (Waddington et al., 1992). However, the concentration of CO_2 decreases at a greater rate than that of O_2 (Waddington et al., 1992). Under normal atmospheric conditions, RuBP binds CO_2 over O_2 at a ratio of 3:1 (Buchanan, 2000). However, when temperatures rise to approximately 30˚C, RuBP carboxylase also has a high affinity to bind O_2 (Fry and Huang, 2004). Oxygen becomes more prevalent in the plant while stomates are closed, as a product of the division of water from the light reaction and the final product from photosynthesis. Once O_2 is bound to RuBP, the Calvin-Benson Cycle continues. However, without enough carbon to form the normal two molecules of 3 PGA; only one molecule of 3 PGA and one molecule of the two-carbon molecule phosphoglycolic acid are formed instead (Wilkins 1984). Phosphoglycolic acid is diverted to another cycle deemed the photorespiratory carbon oxidation cycle (C_2) pathway. This cycle attempts to salvage the lost carbon and transform it to a usable form. The phosphoglycolic acid combines with another molecule of phosphoglycolic acid, forming 3 PGA (Turgeon 2008). The remaining carbon is released as CO_2. For every two molecules of phosphoglycolic acid, the C_2 pathway is able to produce one molecule of 3 PGA and one molecule of CO_2 (Buchanan 2000). Under the C_2 pathway, the plant is forced to release the very molecule it needs to produce energy. The 3 PGA molecule can be sent back to the Calvin-Benson cycle and be used for sugar production, while the CO_2 is generated as waste. As much as half of the CO_2 produced from photorespiration is lost to the atmosphere and the rest can be recycled within the plant (Moore 1998). Since this is an additional cycle to the C_3 pathway, additional energy is required. Under normal atmospheric conditions where CO_2 is bound to RuBP over O_2 at a rate of 3:1, the cost of fixing three CO_2 molecules requires 24 ATP energy unit equivalents, along with 8.25 ATP energy unit equivalents to fix one O_2 molecule, using a total of 32.25 ATP molecules of energy (Buchanan 2000). In contrast, C_4 plants consume 30 ATP energy units to fix three molecules of CO_2. At temperatures within the range of adaptation for C_3 plants, CO_2 concentration within the plant is sufficient enough to prevent the binding of O_2 and the C_2 pathway, making C_3 plants well suited for their environment. As CO_2 concentrations decrease more rapidly relative to oxygen, a greater proportion of energy is spent fixing oxygen, requiring the use of even larger amounts of ATP to produce equivalent amounts of 3 PGA to the C_4 pathway.

Fate of Sugars in Plants

The sugars created from photosynthesis can be used for various things. Cool season grasses store carbohydrates as fructosans and consume them during periods when production is not meeting the consumption demands (Turgeon, 2008). Extremely long chains of carbohydrates are used for cell structure in order to hold it together (Turgeon, 2008). Respiration is the balance for photosynthesis. Whereas photosynthesis uses energy and creates carbohydrates, respiration uses carbohydrates to create energy for the plant. The energy released from respiration is used for growth, metabolism, cell maintenance, nutrient uptake, and transport (Fry, 2004). Unlike photosynthesis, which requires sunlight, respiration can occur at any time of the day or night. The basic reaction associated with respiration:

$$C_6H_{12}O_6 + 6H_2O + 6O_2 \rightarrow 6CO_2 + 12H_2O + \text{Energy}$$

The first stage in respiration is glycolysis and takes place in the cytosol (Moore, 1998). Glycolysis is not dependent on oxygen. However, in order to proceed to the next steps of respiration, oxygen must be available unless the plant is adapted to live in flooded conditions (Campbell et al., 2008). C_3 plants typically store sugar as fructose (Waddington 1992). During glycolysis this six carbon sugar is split to form two 3 carbon molecules of pyruvic acid. The energy released from the breaking of the sugar is used to phosphorylate two ADP into two ATP and reduce two molecules of NAD^+ to two $NADH_2^+$.

Approximately 75% of the energy in the sugar molecule is still contained in the 2 molecules of pyruvic acid (Taiz & Zeiger, 1998). The pyruvic acid leaves the cytosol and enters the mitochondria where the tricarboxylic acid (TCA) cycle takes place. This step is a cycle because the main receiver, OAA, is transformed to citric acid and after several intermediates, OAA is regenerated and the cycle can begin again (Campbell et al., 2008). In order to enter the TCA cycle from glycolysis, the pyruvic acid must lose one CO_2 molecule and one NAD^+ is reduced to $NADH_2^+$ (Campbell et al., 2008). Coenzyme A (CoA) attaches to the newly formed two carbon acetic acid to form acetyl CoA, which can then enter the Krebs cycle (Turgeon, 2008). Once in the Krebs cycle, acetyl CoA binds to the four carbon OAA to form citric acid (Moore, 1998). During the eight-step process of the cycle each molecule of citric acid generates one molecule of ATP, three molecules of $NADH_2+$, and one molecule of $FADH_2$ (Moore, 1998; Taiz & Zeiger, 1998; Buchannan, 2004; Turgeon, 2008; Campbell, 2008). $NADH_2^+$ and $FADH_2$ serve to store energy in electrons and take them to the final phase of respiration known as oxidative phosphorylation.

The third phase of respiration is where the majority of energy is synthesized for the plant to use. The energy from electrons stored in $NADH_2^+$ and $FADH_2$ are relayed to the inner membrane of the mitochondria to a site known as the electron transport chain (Campbell, 2008). Here electron carriers pass the electrons “down” the chain to the more electronegative carrier through a series of oxidative reduction reactions (Campbell, 2008). The electron transport chain’s purpose is to lessen the amount of free energy into several smaller amounts that is more usable to synthesize ATP (Campbell, 2008). As the electrons are being passed down the chain, protons are shuttled from the mitochondrial matrix to the intermembrane space (Moore, 1998). The second step of oxidative phosphorylation is chemiosmosis. During this step, the proton gradient created during the movement of electrons is used to power an ATP synthase. As the protons move back across the gradient, it creates a proton motive force, which the ATP synthase uses to phosphorylate ADP into ATP.

Respiration is the process of the plant transforming sugar into energy. Each hexose sugar molecule can provide 32-38 molecules of ATP (Turgeon, 2008; Campbell, 2008; Moore, 1998; Taiz and Zeiger, 1998). Glycolysis and TCA cycle both contribute 2 substrate level ATP and the oxidative phosphorylation provides 28 to 34 ATP. The exact conversion from $NADH_2^+$ to ATP is not an exact number, but may range from 2.5 to 3.3 and $FADH_2$ to ATP is likely in the range of 1.5 to 2.0 (Campbell, 2008). This accounts for the discrepancy amongst literature. It is generally accepted that respiration produces 36 ATP for each molecule of sugar (Turgeon, 2008). Each ATP molecule holds 7.3 kcal of energy. (Turgeon, 2008; Campbell, 2008). Multiply this number by 36 ATPs to result in 263 kcal of energy created during respiration. As 686 kcal are contained in a molecule of glucose, 72% of the potential energy stored in glucose is lost as heat (Turgeon, 2008).

Surfactants

Surfactants (surface acting agents) are used in a wide variety of situations and are a type of adjuvant. Adjuvants are any material added to a spray solution to increase its performance (Hess, 1999). Adjuvants do not have the regulation that herbicides undergo, therefore the exact composition of the adjuvant is rarely fully disclosed (Hock, 1998; Stock and Briggs, 2000). Classification is therefore sometimes difficult and misunderstood (Hock, 1998; Stock and Briggs, 2000). Surfactants are one chemical classification of adjuvants, along with oil and salts of fertilizers (Hess, 1999), but no one surfactant can perform all adjuvant functions (Hock, 1998). Surfactants aide the spray solution by modifying the emulsifying, dispersing, spreading, sticking or wetting properties of liquids and aide in absorption by changing plant cuticle characteristics (Hess, 1999). Surfactants can be divided into four categories based on their polarity and grouped as non-ionic, anionic, cationic, and amphoteric (Hazen, 2000). Anionic surfactants have a tendency to leach through the soil, while cationic tend to stay bound to soil particles (Turgeon, 2008). Non-ionic surfactants typically have the longest lasting effects, and are thus used most frequently (Turgeon, 2008). Surfactants are composed of a hydrophilic polar group and lipophilic group, which allow it to interact with lipophillic plant surfaces, lipophillic herbicides, hydrophilic herbicides, and water (Hess, 1999). A common misconception is to assume all adjuvants and furthermore surfactants, to be the same (Penner, 2000). It is essential to match the surfactant appropriately to the spray solution based on the solution's characteristics and the target's characteristics (Penner, 2000). One such characteristic is the surfactant's hydrophilic to lipophillic balance (HLB). The HLB value is usually given on a scale of 1-20, with lower numbers being for more lipophillic solutions and higher numbers recommended for more hydrophilic solutions (Hess, 1999). It has been shown that the HLB value of a surfactant applied with an herbicide has had a great effect on the amount of uptake and control (Green and Green, 1992; Nalewaja et al., 1996a; Manthey et al., 1996a; Manthey et al., 1996b; Nalewaja et al., 1996b; Nalewaja et al., 2001). As water solubility of the compound increases, the HLB of the surfactant should also increase to maximize absorption (Stock and Holoway, 1993).

Radiolabeled Compounds

Henri Becquerel discovered radioactivity in 1896. He realized uranium emitted its own source of energy without the aid of the sun (Matis, 2000). After further discoveries and development, the use of radioactive isotopes in biology began in 1923 by Georg Hevesy at the University of Freiburg (Simoni et al., 2002). He used radioactive lead to measure its uptake in plants (Simoni et al., 2002). Every element contains a certain number of protons, neutrons, and electrons (Holden, 2001). These give the element its unique characteristics. Each element has a stable conformation in its proton to neutron ratio (Friedlander et al., 1981). If an element has more or less protons or neutrons than in its stable conformation, it becomes an unstable (or radioactive) element (Friedlander et al., 1981). The element will then try to regain stability by undergoing nuclear reactions (Friedlander et al., 1981). These reactions emit energy that is detectable by certain devices.

Radioactive compounds that contain unstable isotopes, such as $^{14}C_6$, $^{15}N_7$, or $^{3}H_1$, can be inserted into various molecules that can then be used in an experiment as a tracer to record their location (Voges et al., 2009). When inserted in plants, these compounds still react as stable elements would, but also act as tracers moving through the plant (Heidcamp, 1995). The radiolabeled material applied can then be tracked as the compound decays to see how it reacts in the plant (Rennie, 1999). After an allotted time, the plant can be sampled to determine the fate of the compound (Rennie, 1999). If the amount of radioactivity applied is known, then the amount of radioactivity recovered from various sampling methods can determine location of the applied compound.

One technique of measuring radioactivity is to use a liquid scintillation counter (LSC). A LSC measures decay by counting light emitting substances in solution or within a crystal (Heidcamp, 1995). When the radioactive sample is combined with a scintillant molecule, the radiation strikes the scintillant molecule, which will then fluoresce as it re-emits the energy (Heidcamp, 1995). The LSC actually counts the number of flashes. If the amount of radioactivity applied is known, then this measurement not only tells how much is located in the examined spot, but also what percentage of total applied is the examined location. This technique gives very accurate results in accounting for over 90% of applied radiation (Heidcamp, 1995).

The objective of this research was to determine (i) the effects of supplemental sugar applications to heat stressed CBG, (ii) the physiological location of supplemental sugar applications, and (iii) what rate and type of surfactant provided the greatest aid to absorption of fructose.

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CHAPTER II

THE EFFECT OF FRUCTOSE ON CREEPING BENTGRASS PUTTING GREENS

ABSTRACT

Creeping bentgrass being grown out of its hardiness zone is subject to a negative process known as photorespiration in which the plant fails to make sugar from photosynthesis. This results in more intensive labor practices for turf managers to keep the turf alive. Since the plant is failing to make its own sugar, this study investigated the effects of applying sugar in the form of fructose to creeping bentgrass putting greens during the hot summer months. The objective of this study was to determine the effects of foliar fructose applications at alleviating photorespiration on heat stressed creeping bentgrass putting greens. Fructose applied by either Cargill 42 High Fructose Corn Syrup or Swanson 100% Pure Fructose showed no benefit to visual ratings or NDVI measurements. High surfactant rates with the Cargill source resulted in turfgrass injury regardless of fructose rate. Also, low surfactant with high fructose rates resulted in poor turf quality. Turf treated with the Swanson source failed to show to a patterned treatment effect. While it did not result in significant injury like its Cargill counterpart, it also failed to show noticeable improvement on a consistent basis. If either of these sources is providing some benefit at alleviating photorespiration, it was failed to be seen

during the experiment timeframe on the parameters tested. Turf treated with Cargill showed increased clipping yield when compared to Swanson, but this did not translate to improved turf quality or total root length. Cargill had significantly less root length than turf treated with Swanson.

INTRODUCTION

Creeping bentgrass (CBG) (*Agrostis stolonifera* L.) is a fine texture, cool-season turfgrass. It has been used on everything from home lawns to golf courses. However, its dense canopy, fine texture, spreading stolons, and low mowing tolerance, make it the most popular and highest quality choice for golf course fairways and putting greens (Christians, 1998; Beard 2002; Koh et al., 2003; Turgeon, 2008; Cooper and Peacock, 2008). The popularity and publicity of golf has associated CBG putting greens with premier facilities. This demand has resulted in this turf being spread to areas not adapted for its optimal growth (Christians, 1998). When grown in these areas, the turf must receive constant care during the summer months. While CBG does present an excellent putting surface, the amount of labor, chemicals, and irrigation required to keep this surface alive south of the transition zone is insufficient during some years (Dernoeden, 2002).

When C_3 plants, such as CBG, are subjected to extreme stress, it is unable to carry out photosynthesis to its full potential. When C_3 plants are subjected to high temperatures, the plant begins a process known as photorespiration. This efficiency robbing pathway binds O_2 rather than CO_2 at high temperatures, allowing only one molecule of 3-phosphoglyceric acid (3 PGA), the precursor to glucose, to be formed rather than two. As a result, no sugar can be generated and CO_2 is released back into the atmosphere (Turgeon, 2008). Without the main sugar being produced from photosynthesis, respiration levels decrease resulting in slower leaf and root growth from lack of energy.

As the plant is conducting photosynthesis, the production of carbohydrates usually exceeds the use of carbohydrates (Waddington et al., 1992). Cool-season grasses tend to store carbohydrates as long chain polymers of fructose molecules, or fructans (Waddington et al., 1992). However, due to photorespiration, plants do not make sugars and thus have reduced energy stores of fructans to utilize when growing conditions become unfavorable and the plant is unable to conduct photosynthesis.

On golf course putting greens, extensive measures are taken to keep the turf alive. Since most greens are sand based, the water holding capacity is minimal (Beard, 2002). As a result, the greens must be watched on hot afternoons and syringed as needed to prevent desiccation and pest injury (Waddington et al., 1992). Syringing is the process of applying just enough water to wet the leaves without wetting the soil underneath. As the canopy moisture evaporates, a cooling effect results (Dernoeden, 2002). Lowering midday temperatures also helps the turf to reach more optimal, cooler, nighttime temperatures more quickly so it has longer to recuperate (Guertal et al., 2005).

As a precaution against fungal growth, CBG greens are commonly sprayed once a week with a mixture of fungicides and low analysis fertilizers to suppress or prevent fungal growth and encourage growth of the turf. As a way to get water in the root zone and relieve soil compaction, high-pressure water injection or needle tines are used by some superintendents on a regular basis. Wetting agents are another method of getting water to the root zone and reducing hydrophobic nature of soils. Industrial size fans along the border of the greens are sometimes utilized to keep air circulating. Air movement helps cool the heat stressed leaves by reducing turf mat and soil temperatures (Guertal et al., 2005). Management strategies such as these aimed at keeping this putting surface alive results in massive uses of electrical, water, chemical, and labor resources and still may not be able to prevent a decline in turf quality (Guertal et al., 2005).

Little research has been conducted on the application of sugar to various types of plants. Information is limited further on applying sugar to turfgrass. The studies conducted generally have dealt with applying sugar to relieve some kind of stress and improve growth. Reduced light conditions that limit the plant's ability to conduct photosynthesis has been the common stress factor in supplemental sugar studies. Went and Carter (1948) applied a 10% sucrose solution to leaves of tomato plants grown in 23.5 hours of darkness. Plants sprayed with the solution 2.5 hours after daylight exposure (after stomatal closure) grew an average of 83.5 mm, 98.2% greater than control plants. Sucrose applied to plants at the end of the daylight (before stomatal closure) period grew 97.6% more than control plants. Juhren and Went (1948) determined that sugar uptake can occur through leaves of tomatoes even with stomates closed. Juhren and Went (1949) tested applying a 7% sucrose solution to squash (*Cucurbita pepo* L. 'Table Queen') plants grown in darkness. Plants that would typically only live for 4-5 days, would survive for up to 30 days and even grow 50 mm per day (Juhren and Carter, 1949). Berrie (1960) found an increase in dry weight and an increase in development of tomato plants grown in the dark sprayed with a 10% sucrose solution. Sorochan (2002) found a weekly spray of a 1.25% fructose solution to the leaves of supina bluegrass (*Poa supina*) under reduced light conditions demonstrated positive physiological responses relative to the control. Rates higher than 1.25% caused unacceptable leaf injury (Sorochan, 2002). Amiard et al. (2003) applied fructose to *Lolium perenne* L. leaf sheaths following defoliation and found 77% of the sugar incorporated remained in the leaf sheaths, while only 4% and 0.9% was transported to stem and roots, respectively.

Molasses is a similar product that has had some research conducted in agricultural settings, including turf. The difference between molasses and foliar sugar applications is the target of the two products. Molasses is applied in hopes of stimulating soil microbes (Handreck and Black, 2005). This in turn reduces thatch and increases nutrient availability. Molasses is not applied in hopes of directly benefiting the plant but rather as a side effect of increased soil microbe activity (Handreck and Black, 2005).

Heat stress is similar to low light situations in that both result in the plant failing to produce sugar due to a lack of photosynthesis. Therefore, it was hypothesized that exogenous fructose applications could offset the negative effects of photorespiration. Thus, the objective of this study was to determine the effect of fructose applications on turfgrass quality of heat-stressed CBG Results from this study could be a cost effective measure for golf course superintendents to fight summer decline of CBG due to heat stress.

MATERIALS AND METHODS

Putting Green Test

The bentgrass putting green was installed on Mississippi State University's R.R. Foil Plant Science Research Center in the fall of 2007. A 232.25 m^2 square plot was seeded with three cultivars/species of bentgrass. This study was conducted on a 77.42 m^2 section of 'A4' CBG. The bentgrass was maintained at golf green conditions consisting of mowing 5 times per week at 0.32 cm. Various forms of nitrogen were applied at 12.21 kg ha^{-1} monthly. Phosphorous and K were applied at 4-8 kg ha^{-1} monthly. Fungicide applications were made as needed. A soil test conducted on 16 May 2008 showed the soil pH to be 7.3. Nutrient levels were as follows: P 9 kg ha^{-1}, K 34.7 kg ha^{-1}, Mg 52.7 kg ha^{-1}, Zn 1.2335 kg ha^{-1}, and Ca 598.81 kg ha^{-1}. The growing medium was a United States Golf Association specification, 90:10 mix of sand to reed sedge peat. A sand topdressing was applied at 0.317cm monthly during the summer. Irrigation to replace evapotranspiration was supplied every three days. Supplemental syringing applications of water to reduce overheating and death of plant were applied as needed.

Treatments - 2008

Within the study area, 64 plots measuring 91 x 121.9 cm were marked off for various treatment levels. The study consisted of 3 reps of 21 treatments in a randomized complete block design with the remaining plot being an extra control plot. Four of the 64 plots were untreated controls with no treatments applied. The remaining 60 plots received a combination of a level of sugar and a level of surfactant. The treatments were based on a v/v ratio (Table 2.1). Berrie (1960) found it impossible to increase spray of sucrose above 10% solution due to excessive crystallization on leaves and stem. There were two levels of surfactant applied with the sugar, a high and a low level. The low level is a 0.25% v/v and the high level is a 1.0% v/v (Table 2.1). Cargill ™ IsoClear ® 42% High Fructose Corn Syrup (Cargill Inc., Minneapolis, MN) was used as the sugar source and Hi-Yield™ Spreader Sticker (Voluntary Purchasing Group, Bonham, TX) was used as the surfactant. Due to limited space, only one source of each item could be tested.

Table 2.1 Treatment levels for 2008 applications of Cargill 42 High Fructose Corn Syrup.

Treatment Number	Rate of Sugar[1] (v/v)	Rate of Surfactant[2]
1	0	0
2	0.00%	Low
3	0.25%	Low
4	0.50%	Low
5	0.75%	Low
6	1.00%	Low
7	1.50%	Low
8	2.00%	Low
9	4.00%	Low
10	6.00%	Low
11	8.00%	Low
12	0.00%	High
13	0.25%	High
14	0.50%	High
15	0.75%	High
16	1.00%	High
17	1.50%	High
18	2.00%	High
19	4.00%	High
20	6.00%	High
21	8.00%	High

[1]Rate of sugar is based on a volume to volume percentage.
[2]Rate of surfactant is based on a nominally high and low rate of surfactant. High = 1.0% (v/v) and Low = 0.25% (v/v).

Treatments - 2009

Due to inconsistent results, a different source of fructose as well as surfactant were selected in 2009. The Cargill source was only 42% fructose, therefore the other 58% made up of dextrose (52%), maltose (3%), and higher saccharides (3%), might have been contributing to the negative effects experienced. Thus, Swanson Health Products 100% Pure Fructose (Swanson Health Products, Fargo, ND) was used as the fructose source and Southern Ag Surfactant for Herbicides (Southern Agriculture Insecticides Inc., Palmetto, FL) as the surfactant. The 2009 study consisted of fewer treatments as a result of eliminating the 0.25%, 0.75%, and 1.5% rate of fructose from both surfactant levels since no significant difference was seen between these treatments and other treatments with similar fructose levels and to allow larger plots for observation. The study area was divided into 45 plots measuring 91.44 x 152.4 cm. The study consisted of 3 reps of 15 treatments in a randomized complete block design. Treatments for 2009 are shown in Table 2.2. The treatments are based on a mass to volume ratio. There were two levels of surfactant applied with the sugar, a high and a low level. The low level was a 0.25% v/v and the high level was a 1.0% v/v.

Table 2.2 Treatment levels for 2009 applications of Swanson 100% Pure Fructose.

Treatment Number	Rate of Sugar[1] (m/v)	Rate of Surfactant[2]
1 (Control)	0	0
2	0.00%	Low
3	0.50%	Low
4	1.00%	Low
5	2.00%	Low
6	4.00%	Low
7	6.00%	Low
8	8.00%	Low
9	0.00%	High
10	0.50%	High
11	1.00%	High
12	2.00%	High
13	4.00%	High
14	6.00%	High
15	8.00%	High

[1]Rate of sugar is based on a volume to volume percentage.
[2]Rate of surfactant is based on a nominally high and low rate of surfactant. High = 1.0% (v/v) and Low = 0.25% (v/v).

Application

The combination of sugar and surfactant was contained in 2 liter bottles and applied via a CO_2 backpack sprayer maintained at 206.8 kPa. Each plot received the assigned treatment in a carrier of 200 mL of water. A 2 nozzle handheld boom provided uniform coverage over the plots. Applications were done on a weekly basis in the early morning before temperatures rose in an attempt to apply the treatments before stomatal closure.

Data Measurement

For the 2008 and 2009 study, two methods of measuring turf quality were implemented. The first was a visual rating of turf quality and the second was a Normalized Difference Vegetative Index (NDVI) of turf color. The visual ratings were taken once a week on the day after treatment application using a 1-9 scale (where 1 = brown, dead turf, 9 = exceptional, dark green turf, and 7 = minimal acceptable quality for golf course putting greens). Also on the first or second day after application a reading was taken using a GreenSeeker® (NTech Industries, Ukiah, CA) handheld device to measure Normalized Difference Vegetative Index (NDVI). The device can be used to monitor plant conditions during the growing season and the effects of different levels of a treatment compared to a control plot (Anonymous A, 2009). The device measures the wavelengths and intensity of visible and near infrared light reflected by the plant (Weier and Herring, 2010). For instance, a healthy plant's chloroplasts will absorb most of the visible light that strikes it while it reflects a larger portion of the near infrared light (Weier and Herring, 2010). An unhealthy or less dense plant will reflect more of the visible light and absorb more near infrared light (Weier and Herring, 2010). The device then uses an algorithm to quantify the green intensity on a 0 – 1 scale. The formula described by Weier and Herring (2010) can be represented by:

$$NDVI = \frac{(\text{Near Infrared} - \text{Visible})}{(\text{Near Infrared} + \text{Visible})}$$

During the 2009 study a third data measurement was added by taking root samples once a month. This was done by taking three soil probes measuring 1.905cm in diameter and 20.32cm in length from each plot to measure the longest root found in each of the probes to monitor changes in root length. Data could not be taken more frequently due to the destructive nature of the sampling potentially having negative effects on visual ratings and NDVI readings.

Data were analyzed using the general linear model procedure of the Statistical Analysis Software (SAS Institute 9.2, Cary, NC). Mean separation was conducted using Fisher's protected LSD with an alpha level of 0.05.

Glasshouse Study - 2010

Since two fructose sources were used for field studies each of the previous two years, a second year of data for each source was needed. Due to time, weather, and space constraints it was decided to conduct this in the glasshouse located on the campus of Mississippi State University. The number of treatment levels was reduced due to lack of differences from previous year's treatments and space constraints in the glasshouse. Treatment levels for the Cargill 42 High Fructose Corn Syrup and Swanson 100% Pure Fructose can be found in Table 2.3. Plugs of turf from the same research area from previous summers were obtained using a 4" diameter golf course cup cutter (Standard Golf, Cedar Falls, IA). Plugs were placed in a 10.16cm diameter and 30.48cm tall PVC pipe. The lysimeters themselves were capped on one end with holes for drainage. The pipe was lined with paper towels and filled with a 90% sand, 10% peat mixture up to the base of plug. The plug was inserted into the pipe so the crown of the turf was level with the top of the pipe. Each treatment was replicated 3 times for a total of 39 pipes for each fructose source in a randomized complete block design. Pipes were placed in the glasshouse and maintained at 32±3 ˚C. Plants were watered daily until water came out of the bottom of the column and this was assumed to be “saturation.” Fertilizer was applied on a weekly basis at a rate of 12.21kg ha^{-1} of nitrogen monthly. Phosphorous and potassium were applied at 4-8 kg ha^{-1} monthly. Pots were mowed weekly at a height of 0.32 cm with scissors.

Application

Fructose treatments were applied in the same method as previous summers. Pipes from the same treatment level were placed in a marked out area of the same dimensions as previous plots. Treatments were applied using 2-Liter bottles and a CO_2 backpack sprayer maintained at 206.84 kPa. The treatment was applied over the designated area equally so the pots received similar amounts of fructose as the previous studies.

Table 2.3 Treatment levels for 2010 applications of Cargill 42 High Fructose Corn Syrup and Swanson 100% Pure Fructose in glasshouse setting.

Treatment Number	Rate of Sugar [1](v/v)	Rate of Surfactant[2]
1	0	0
2	0.50%	Low
3	1.00%	Low
4	2.00%	Low
5	4.00%	Low
6	6.00%	Low
7	8.00%	Low
8	0.50%	High
9	1.00%	High
10	2.00%	High
11	4.00%	High
12	6.00%	High
13	8.00%	High

[1]Rate of sugar is based on a volume to volume percentage.
[2]Rate of surfactant is based on a nominally high and low rate of surfactant. High = 1.0% (v/v) and Low = 0.25% (v/v).

Data Measurement

Data measurements consisting of visual ratings and NDVI readings were taken in similar methods as previous years. Visual ratings were taken the day after application using a 1-9 scale (where 1 = brown, dead turf, 9 = exceptional, dark green turf, and 7=minimal acceptable turf quality for golf course putting greens) to measure turfgrass quality. Normalized Difference Vegetative Index (NTech Industries Ukiah, CA) readings were taken on the day after application.

Adjustments were made in taking readings to account for the small surfece areas of the pot. The NDVI meter was mounted on a wheeled cart to allow for a consistent height and stable readings. A black photography cloth was laid on the ground to give a uniform background. Pots were placed one at a time in the center of the cloth. The NDVI meter was wheeled over the pot until out of range and then wheeled backwards over the pot, so two scans per pot were obtained. The NDVI meter takes about 15 readings per second and cart travel took approximately 5 seconds. The majority of readings involved the black background, which gave a score of near zero. The top 10 readings were taken as the lysimeters actual reading and averaged to give the NDVI for each pot. A new data measurement was implemented for the glasshouse study that was not as feasible to get accurate in the field. Clipping yield data began at week 5 of treatments to see differences in mass of clippings. This was delayed due to clippings being needed for a supplementary test. Pots were mowed once a week using scissors to a height of 0.32cm. Clippings were collected and then oven dried at 71˚C for one week and then weighed. The final method of data analysis for the glasshouse study was root length measurements. Due to the limited size of the pots and the destructive nature of root sampling, this could not be done as it was for the summer 2009 study. Instead samples could only be taken once at the end of the glasshouse study. The entire plug of turf was removed from the pot and shaken to remove loose sand. The longest root was measured and recorded as the pot's root length. The same two methods of measuring turf quality and color in the field were implemented in this trial.

Data were analyzed using the general linear model procedure of the Statistical Analysis Software (SAS Institute 9.2, Cary, NC). Mean separation was conducted using Fisher's protected LSD with an alpha level of 0.05.

RESULTS

Cargill Fructose Source

Turf Quality Ratings

Throughout the Cargill fructose treatments, significant differences in visual ratings occurred among treatments (Figure 2.1, Figure 2.2). The initial visual rating conducted before treatments began both years showed no significant difference among treatments. No treatment consistently improved turf quality of the course of either year.

For 2008 high fructose and surfactant levels typically resulted in the lowest turf quality ratings. The majority of low level surfactant treatments had acceptable turf quality, but not significantly better than the control, leaving it unsubstantiated whether or not foliar applied fructose provided any visible benefit to turf quality under the conditions experienced. The highest three rates of fructose from this source resulted in unacceptable turfgrass quality regardless of surfactant level. At fructose rates between 0.5 and 2%, coupled with the low surfactant rate, turfgrass quality was acceptable, but not better than the untreated control.

For 2010 all fructose treatments accompanied with the high surfactant resulted in unacceptable turf quality ratings (< 7.0). All pots receiving the low rate of surfactant, regardless of fructose treatment, had a higher average turf quality than the control and met the minimally acceptable rating. Further, they were all significantly better than high surfactant treatments.

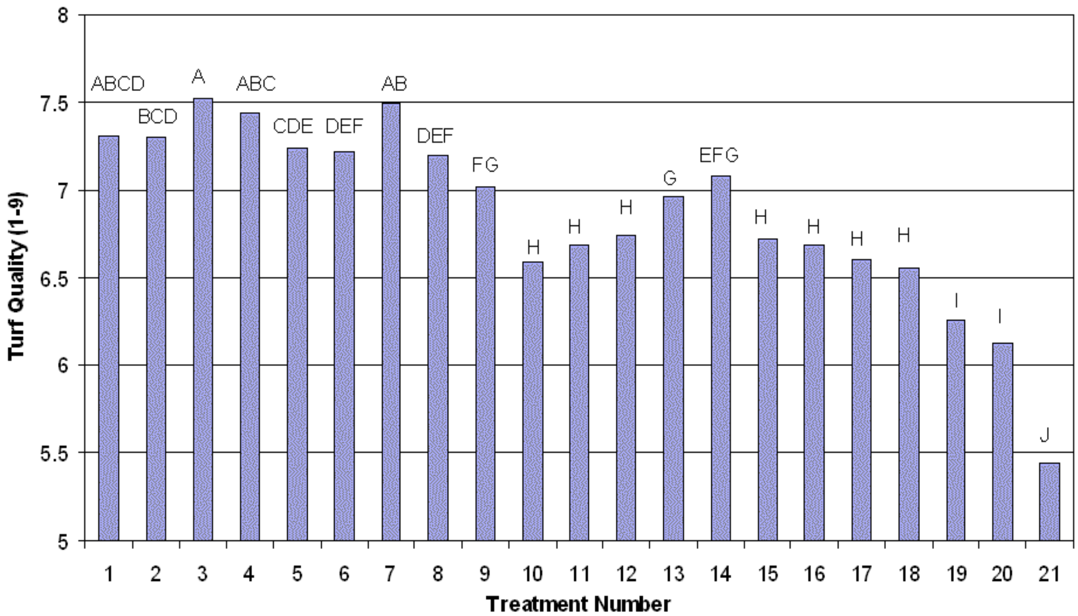

Figure 2.1. Turf quality visual ratings for plots treated with Cargill 42 High Fructose Corn Syrup during summer 2008.

Turf Quality was rated on a 1 to 9 scale, 1 = brown, dead turf, 7 was the minimum value for acceptable golf course putting green turf quality, 9 = ideal turf. Treatment number is equal to 1 = Control, 2 = 0.00% L, 3 = 0.25% L, 4 = 0.50% L, 5 = 0.75% L, 6 = 1.00% L, 7 = 1.50%, 8 = 2.00% L, 9 = 4.00% L, 10 = 6.00% L, 11 = 8.00% L, 12 = 0.00% H, 13 = 0.25% H, 14 = 0.50% H, 15 = 0.75% H, 16 = 1.00% H, 17 = 1.50% H, 18 = 2.00% H, 19 = 4.00% H, 20 = 6.00% H, 21 = 8.00% H; where L (low surfactant) = 0.25% and H (high surfactant) = 1.00%. Hi-Yield™ Spreader Sticker was used as surfactant source. Different letters above treatment number bar indicate significant difference at the 0.05 level (P<0.05).

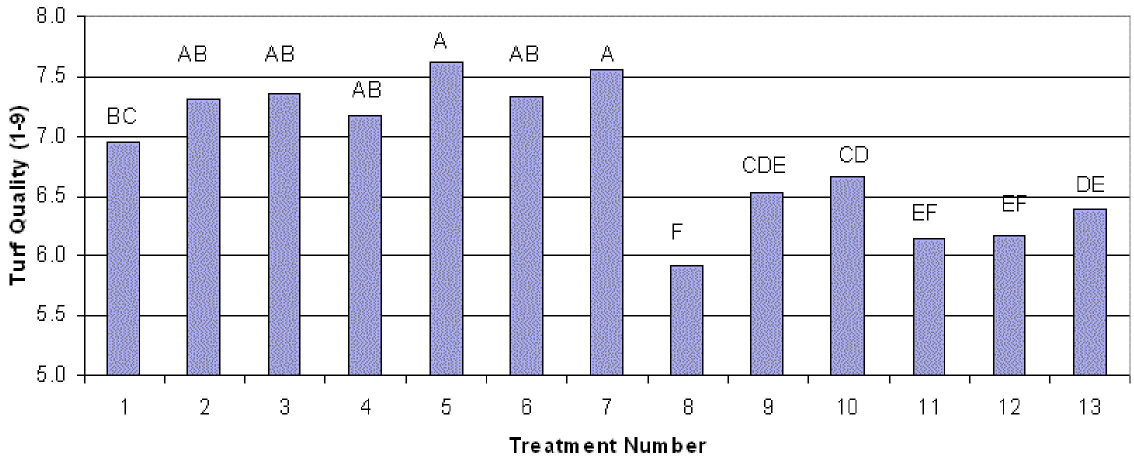

Figure 2.2. Turf quality visual ratings of pots in glasshouse treated with Cargill 42 High Fructose Corn Syrup during 2010.

Turf Quality was rated on a 1 to 9 scale, 1 = brown, dead turf, 7 was the minimum value for acceptable golf course putting green turf quality, 9 = ideal turf. Treatment number is equal to 1 = Control, 2 = 0.50% L, 3 = 1.00% 4 = 2.00% L, 5 = 4.00% L, 6 = 6.00% L, 7 = 8.00% L, 8 = 0.50% H, 9 = 1.00% H, 10 = 2.00% H, 11 = 4.00% H, 12 = 6.00% H, 13 = 8.00% H; where L (low surfactant) = 0.25% and H (high surfactant) = 1.00%. Hi-Yield™ Spreader Sticker was used as surfactant source. Different letters above treatment number bar indicate significant difference at the 0.05 level (P<0.05).

Date was highly significant in regards to turf quality for both years (Table 2.4). For 2008, turf visual ratings showed a general upward trend for the first seven weeks until daily syringe irrigation was reduced from eight 1 min 20 sec cycles to four 1 min 20 sec cycles in order to increase heat stress. Visual ratings then showed the lowest average, remaining low until high temperatures subsided during the final week of observation. For 2010, turf quality trends remained relatively steady over the first 8 weeks. During week 9, however, quality significantly decreased to the lowest level and remained at this level for the duration of the study.

Table 2.4 Mean turf quality ratings by week for 2008, 2009, & 2010 across all treatments.

WAT	2008[3]	2009[4]	2010-C[5]	2010-S[6]
	--------------------------Turf Quality[1] (1-9)-------------------------			
1	6.70 def	7.09 bc	7.13 abc	6.69 e
2	6.86 cd	7.13 bc	7.13 abc	6.85 cde
3	6.78 cde	7.42 a	7.49 a	7.03 c
4	6.89 bcd	6.91 dc	7.39 ab	7.28 ab
5	7.08 abc	6.96 dc	6.92 c	6.97 cd
6	6.61 def	7.11 bc	6.97 bc	6.85 cde
7	7.23 a	6.22 g	6.87 c	6.77 de
8	6.44 f	6.51 ef	7.21 abc	6.82 cde
9	6.49 ef	6.71 de	6.21 d	6.87 cde
10	6.57 def	6.42 fg	6.18 d	6.67 e
11	6.52 ef	6.96 dc	6.39 d	7.05 bc
12	7.17 ab	7.27 ab	6.28 d	7.36 a
Mean[7]	6.78	6.89	6.93	6.85

[1]Turf Quality was rated visually on a 1 to 9 scale, 1 = brown, dead turf, 7 was the minimum value for acceptable golf course putting green turf quality, 9 = ideal turf.
[2]Weeks after initial treatment.
[3]2008 study used Cargill 42 High Fructose Corn Syrup as fructose source applied weekly beginning July 15th and ending October 2nd.
[4]2009 study used Swanson 100% Pure Fructose as fructose source applied weekly beginning June 3rd and ending August 24th.
[5]2010-C study used Cargill 42 High Fructose Corn Syrup as fructose source applied weekly beginning January 28th and ending April 20th.
[6]2010-S study used Swanson 100% Pure Fructose as fructose source applied weekly beginning January 28th and ending April 20th.
[7]Mean corresponds to average turf quality across all weeks within a source. Different letters within the same column indicate significance at the 0.05 level ($P<0.05$).

NDVI Readings

Normalized Difference Vegetative Index readings were also taken weekly either the first or second day after treatment application. In 2008, the NDVI meter was not acquired until after the first application of fructose had been applied, therefore, no reading was available to test for initial variation among plots. Visual ratings at that time showed no significant difference among plots. Over the course of both years, NDVI readings detected significant differences between treatments (Figure 2.3, Figure 2.4). No treatment was found to consistently have the highest NDVI measurements.

For 2008, high levels of sugar and surfactant resulted in the lowest NDVI readings and appear to be unsuitable for foliar applications to A4 CBG. The rate of high fructose corn syrup seems to be the determining factor in the level of phytotoxicity with the rate of surfactant exacerbating the damage at the highest rate of sugar.

For 2010 no treatment was significantly better than the control. The control and all low surfactant treatments had a higher average NDVI score than treatments receiving the high rate of surfactant. The highest 3 rates of sugar and surfactant yielded the lowest NDVI readings.

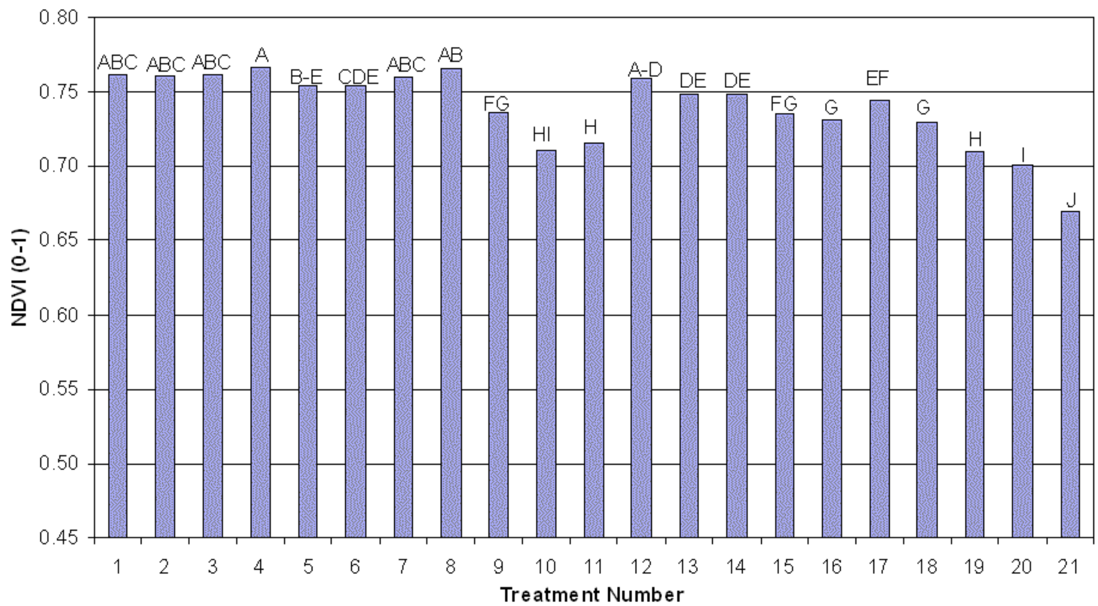

Figure 2.3 NDVI color ratings of plots treated with Cargill 42 High Fructose Corn Syrup for summer 2008.

NDVI – Normalized Difference Vegetative Index which assesses the amount of live green material in the sampling area where 0 = no green material and 1 = ideal turf. Treatment number is equal to 1 = Control, 2 = 0.00% L, 3 = 0.25% L, 4 = 0.50% L, 5 = 0.75% L, 6 = 1.00% L, 7 = 1.50%, 8 = 2.00% L, 9 = 4.00% L, 10 = 6.00% L, 11 = 8.00% L, 12 = 0.00% H, 13 = 0.25% H, 14 = 0.50% H, 15 = 0.75% H, 16 = 1.00% H, 17 = 1.50% H, 18 = 2.00% H, 19 = 4.00% H, 20 = 6.00% H, 21 = 8.00% H; where L (low surfactant) = 0.25% and H (high surfactant) = 1.00%. Hi-Yield™ Spreader Sticker was used as surfactant source. Different letters above treatment number bar indicate significant difference at the 0.05 level ($P<0.05$).

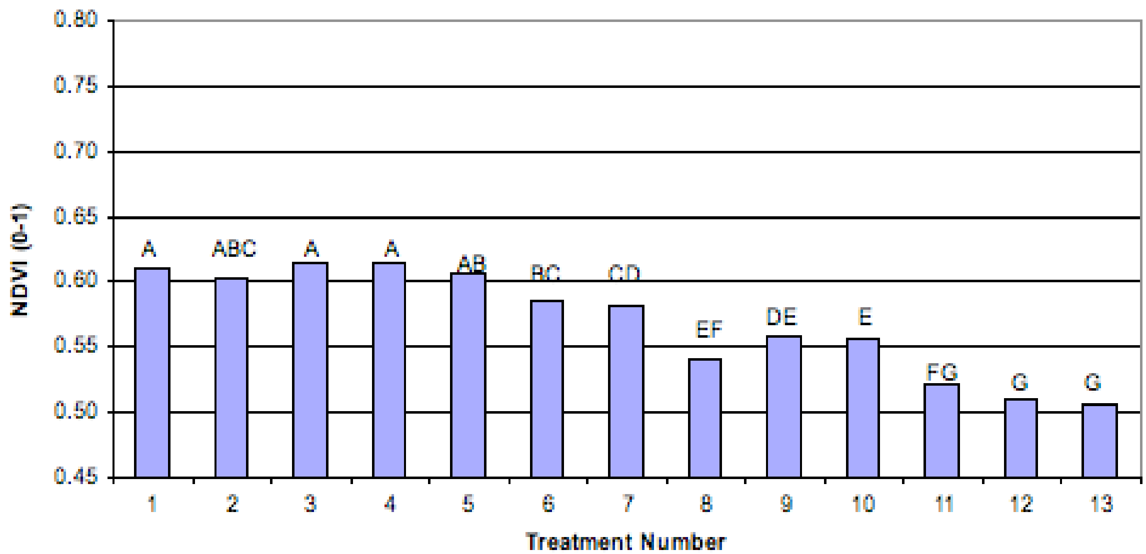

Figure 2.4 NDVI measurements for pots treated with Cargill 42 High Fructose Corn Syrup in glasshouse of 2010.

NDVI – Normalized Difference Vegetative Index which assesses the amount of live green material in the sampling area where 0 = no green material and 1 = ideal turf. Treatment number is equal to 1 = Control, 2 = 0.50% L, 3 = 1.00% 4 = 2.00% L, 5 = 4.00% L, 6 = 6.00% L, 7 = 8.00% L, 8 = 0.50% H, 9 = 1.00% H, 10 = 2.00% H, 11 = 4.00% H, 12 = 6.00% H, 13 = 8.00% H; where L (low surfactant) = 0.25% and H (high surfactant) = 1.00%. Hi-Yield™ Spreader Sticker was used as surfactant source. Different letters above treatment number bar indicate significant difference at the 0.05 level (P<0.05).

Statistical analysis showed significant difference in regards to NDVI analysis and date for both years (Table 2.5). For 2008, week 7 and 10 were the significant best and worst respectively. Aside from a dip in week 4, increases were experienced from weeks 3-8. Weeks 8-10 saw decreases in NDVI readings that were associated with decreased watering regiment. The final two weeks saw an increase in NDVI, which was likely associated with the cooler nighttime temperatures (average of 6° C cooler than previous two weeks). For 2010, week 9 had the lowest NDVI rating during that year. However, there was no single week that clearly stood out as having the highest NDVI. Readings remained high for the first few weeks and then trended downward to the lowest level in week 9. The last three weeks saw a gradual increase, but was still significantly less than the control.

Table 2.5 Mean NDVI readings by week for 2008, 2009, & 2010 across all treatments.

WAT[2]	2008[3]	2009[4]	2010-C[5]	2010–S[6]
	-------------------------------------NDVI [1]----------------------------------			
1	------------	0.735 d	0.666 a	0.656 c
2	0.740 c	0.736 cd	0.677 a	0.695 b
3	0.755 b	0.750 b	0.660 a	0.729 a
4	0.720 e	0.705 f	0.617 c	0.720 a
5	0.731 d	0.714 e	0.537 de	0.639 d
6	0.746 c	0.781 a	0.512 f	0.585 f
7	0.822 a	0.746 b	0.543 de	0.615 e
8	0.760 b	0.743 bc	0.521 ef	0.584 g
9	0.697 f	0.781 a	0.455 g	0.590 f
10	0.669 g	0.744 bc	0.527 ef	0.538 g
11	------------	0.716 e	0.552 cd	0.620 e
12	0.756 b	0.777 a	0.573 c	0.645 cd
Mean[7]	0.740	0.744	0.570	0.635

[1]NDVI – Normalized Difference Vegetative Index which assesses the amount of live green material in the sampling area where 0 = no green material and 1 = ideal turf.
[2]Week after initial treatment.
[3]2008 study used Cargill 42 High Fructose Corn Syrup as fructose source applied weekly beginning July 24th and ending October 6th.
[4]2009 study used Swanson 100% Pure Fructose as fructose source applied weekly beginning June 3rd and ending August 24th.
[5]2010-C study used Cargill 42 High Fructose Corn Syrup as fructose source applied weekly beginning January 28th and ending April 20th.
[6]2010-S study used Swanson 100% Pure Fructose as fructose source applied weekly beginning January 28th and ending April 20th.
[7]Mean corresponds to average NDVI readings across all weeks within a source. Different letters within the same column indicate significance at the 0.05 level ($P<0.05$).

Root Measurements

Significant differences were not found within Cargill treatments for 2010. Since the measurement was of a destructive nature, root measurements could only be taken once, therefore date could not be analyzed.

Clipping Measurements

Significant differences were found among treatments and date (Figure 2.5, Figure 2.6). However, there was no single treatment that clearly stood out as having the highest or lowest dry mass of clippings. All high surfactant pots had a lower average than pots receiving low surfactant across all fructose rates. The general trend for low-level surfactant treatments indicates as fructose level increased, clipping yield decreased. An initial increase in clipping yield over the first three weeks could be indicative of a positive treatment response. However, the final 5 weeks showed significant decreases, finishing with its lowest level.

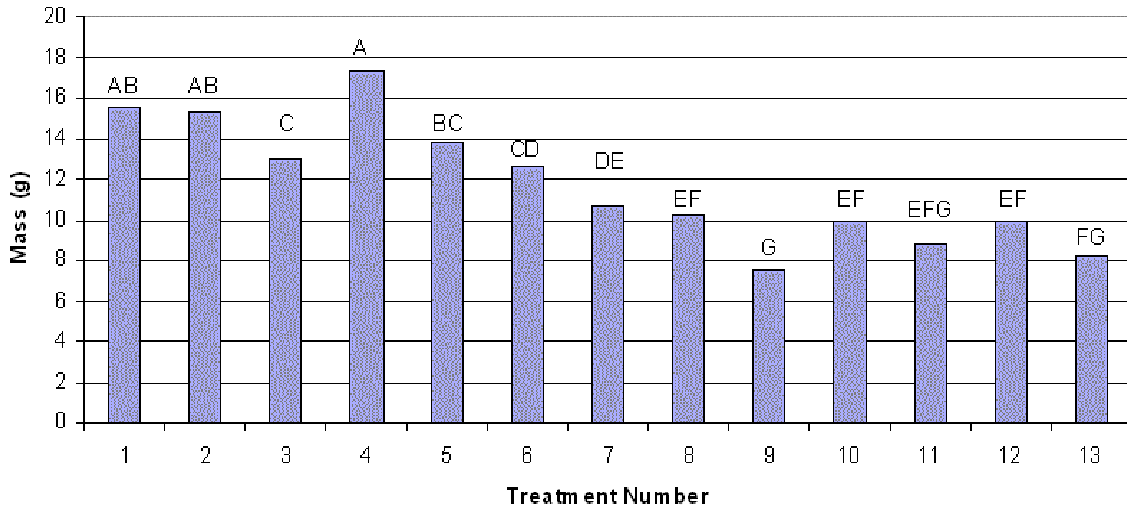

Figure 2.5 Clipping mass yield by treatment for pots treated with Cargill 42 High Fructose Corn Syrup.

Treatment number is equal to 1 = Control, 2 = 0.50% L, 3 = 1.00% 4 = 2.00% L, 5 = 4.00% L, 6 = 6.00% L, 7 = 8.00% L, 8 = 0.50% H, 9 = 1.00% H, 10 = 2.00% H, 11 = 4.00% H, 12 = 6.00% H, 13 = 8.00% H; where L (low surfactant) = 0.25% and H (high surfactant) = 1.00%. Hi-Yield™ Spreader Sticker was used as surfactant source. Different letters above treatment number bar indicate significant difference at the 0.05 level (P<0.05).

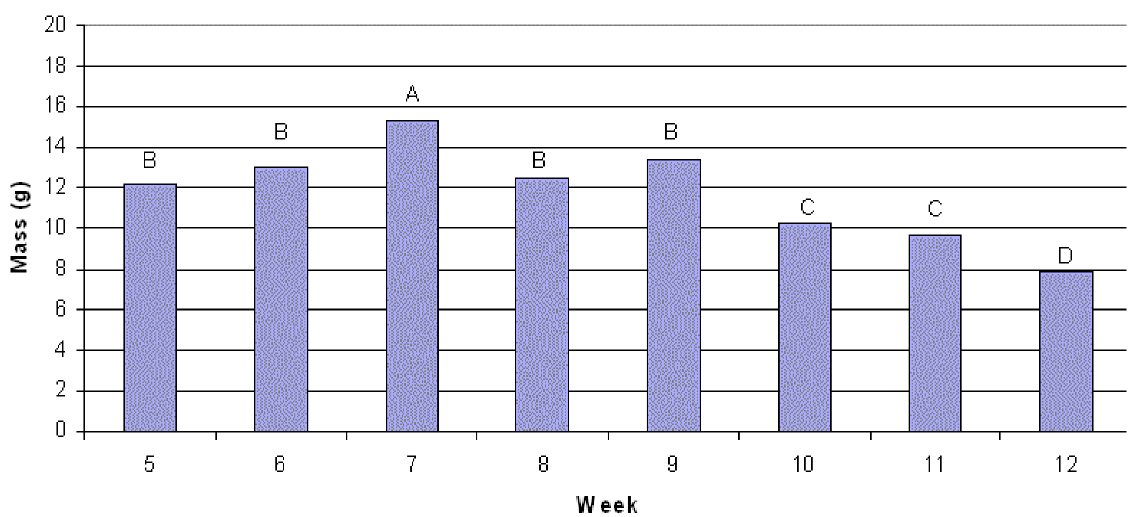

Figure 2.6 Clipping mass yield by week for pots treated with Cargill 42 High Fructose Corn Syrup.

Different letters above treatment number bar indicate significant difference at the 0.05 level (P<0.05).

Swanson Fructose Source

Turf Quality Ratings

Swanson applications resulted in less than one point of separation from the highest to the lowest quality rating for both years. No significant differences were found within a single rating date. When averaged over the entire summer significant quality differences did occur across treatments, however there appears to be no real pattern to the results in relation to treatment (Figure 2.7, Figure 2.8).

For 2009, high and low levels of surfactant were interspersed throughout the ratings with no relation to level of fructose. Further, the level of fructose appeared to have very little impact on visual rating. For instance, the treatment with the second highest level of sugar and high surfactant produced the lowest average visual rating whereas the treatment with the highest level of sugar and surfactant was among the best. Further, the two treatments (8 & 9) with the highest average turf quality, and the only treatments significantly higher than the control plot had no similarities in fructose/surfactant rate.

For 2010 five of the treatments as well as the control were found to have acceptable turf quality. No treatment was found to be consistently the highest or lowest. No treatment was significantly better than the control. There appeared to be no distinction among treatments with high level of surfactant and low level of surfactant, with 2 of the 5 acceptable turf quality treatments receiving the high level of surfactant.

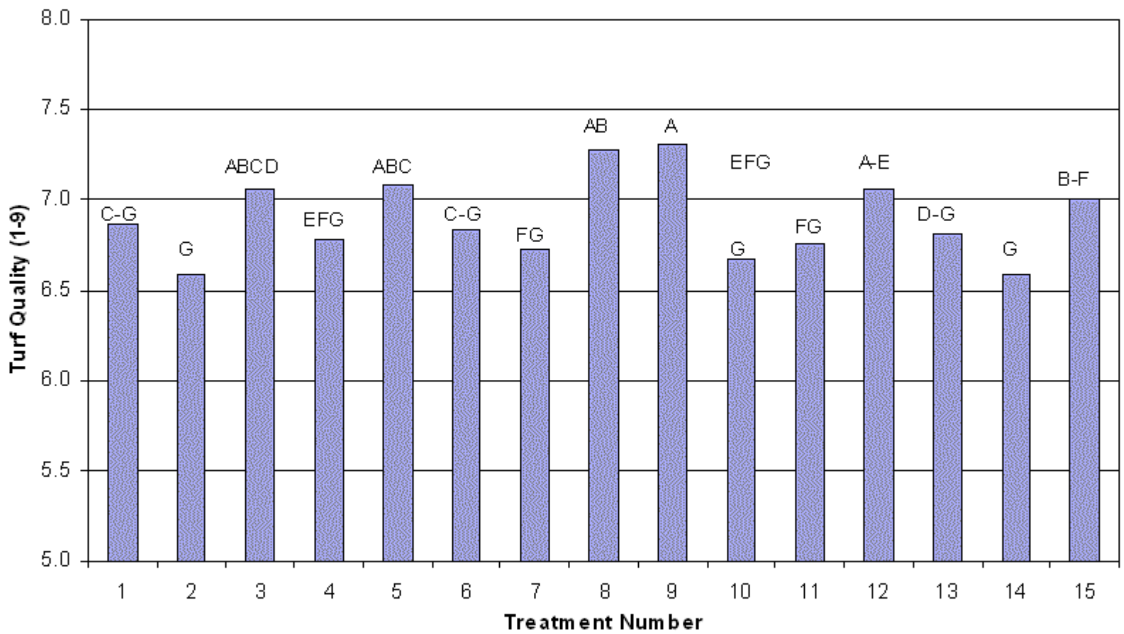

Figure 2.7 Turf Quality visual ratings of plots treated with Swanson 100% Pure Fructose for summer 2009.

Turf Quality was rated on a 1 to 9 scale, 1 = brown, dead turf, 7 was the minimum value for acceptable golf course putting green turf quality, 9 = ideal turf. Treatment number is equal to 1 = Control, 2 = 0.00% L, 3 = 0.50% L, 4 = 1.00% 5 = 2.00% L, 6 = 4.00% L, 7 = 6.00% L, 8 = 8.00% L, 9 = 0.00% H, 10 = 0.50% H, 11 = 1.00% H, 12 = 2.00% H, 13 = 4.00% H, 14 = 6.00% H, 15 = 8.00% H; where L (low surfactant) = 0.25% and H (high surfactant) = 1.00%. Southern Ag Surfactant for Herbicides was used as surfactant source. Different letters above treatment number bar indicate significant difference at the 0.05 level ($P<0.05$).

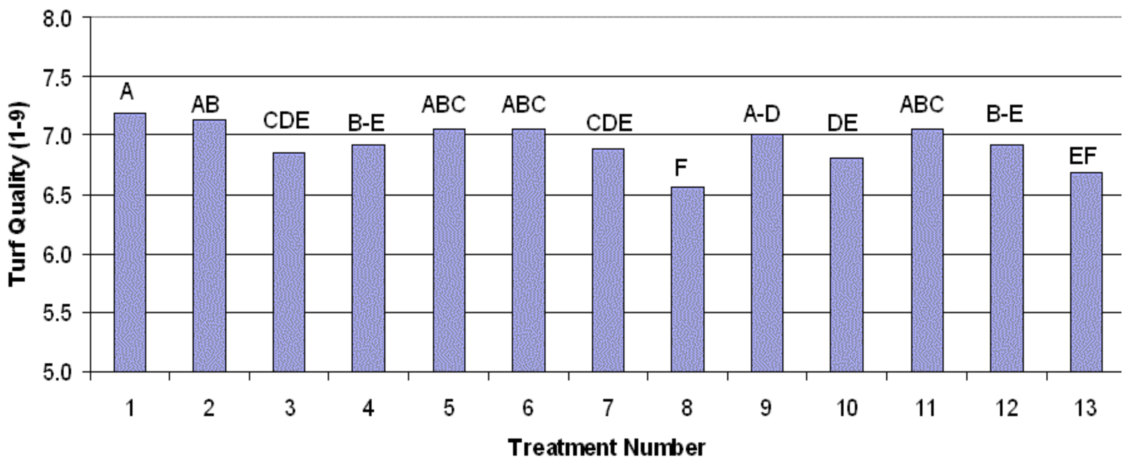

Figure 2.8 Visual ratings of 'pots treated with Swanson 100% Pure Fructose for glasshouse 2010.

Turf Quality was rated on a 1 to 9 scale, 1 = brown, dead turf, 7 was the minimum value for acceptable golf course putting green turf quality, 9 = ideal turf. Treatment number is equal to 1 = Control, 2 = 0.50% L, 3 = 1.00% 4 = 2.00% L, 5 = 4.00% L, 6 = 6.00% L, 7 = 8.00% L, 8 = 0.50% H, 9 = 1.00% H, 10 = 2.00% H, 11 = 4.00% H, 12 = 6.00% H, 13 = 8.00% H; where L (low surfactant) = 0.25% and H (high surfactant) = 1.00%. Southern Ag Surfactant for Herbicides was used as surfactant source. Different letters above treatment number bar indicate significant difference at the 0.05 level (P<0.05).

Statistical analysis showed date to be significant in regards to turf quality (Table 2.4). However, there was no single date that clearly stood out as having the highest or lowest turf quality. Turf quality followed an up and down trend over the course of both study years by increasing initially, then decreasing for a majority of the weeks, then increasing again.

NDVI Readings

Significant differences occurred among treatments, although no consistent trends could be identified (Figure 2.9, Figure 2.10). No significant differences occurred within a single rating day in regards to treatment. When averaged over the entire study, significant differences occurred among treatments, but the differences did not appear to be related to fructose treatment. This is evidenced further by the fact that positions in rank between years had no real similarities. High and low levels of sugar were both dispersed throughout the rankings. Ranking of treatment effects showed differing results, failing to demonstrate a pattern. Week was found to be significant in regards to NDVI readings (Table 2.5). However, there was no single week that clearly stood out as having the highest NDVI reading. Normalized Difference Vegetative Index followed an up and down trend over the course of both study years.

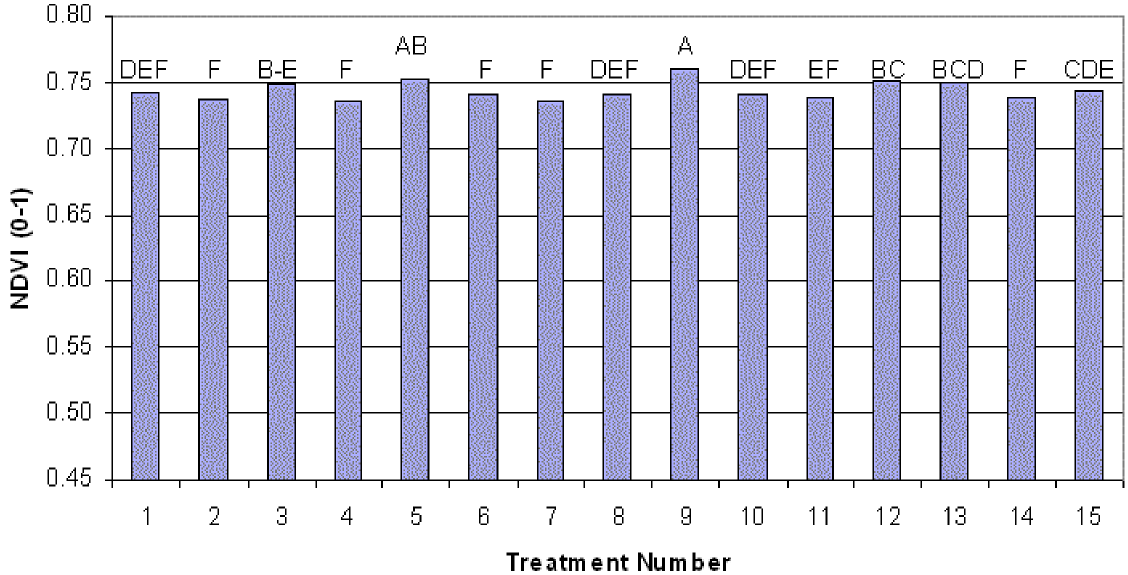

Figure 2.9 NDVI ratings of plots treated with Swanson 100% Pure Fructose for summer 2009.

NDVI – Normalized Difference Vegetative Index, which assesses the amount of live green. material in the sampling area where 0 = no green material and 1 = ideal turf. Treatment number is equal to 1 = Control, 2 = 0.00% L, 3 = 0.50% L, 4 = 1.00% 5 = 2.00% L, 6 = 4.00% L, 7 = 6.00% L, 8 = 8.00% L, 9 = 0.00% H, 10 = 0.50% H, 11 = 1.00% H, 12 = 2.00% H, 13 = 4.00% H, 14 = 6.00% H, 15 = 8.00% H; where L (low surfactant) = 0.25% and H (high surfactant) = 1.00%. Southern Ag Surfactant for Herbicides was used as surfactant source. Different letters above treatment number bar indicate significant difference at the 0.05 level (P<0.05).

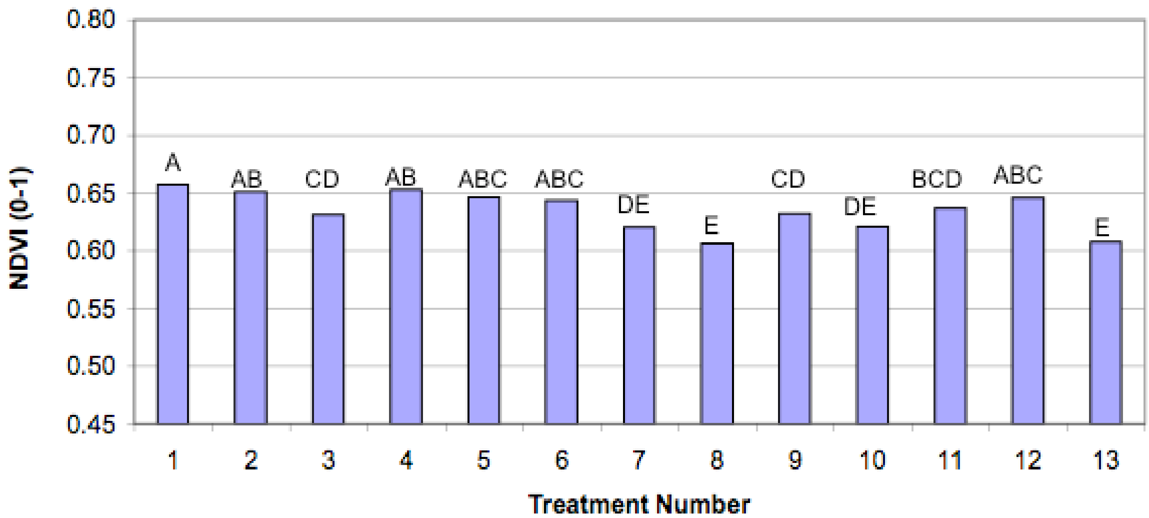

Figure 2.10 NDVI measurements of pots treated with Swanson 100% Pure Fructose for glasshouse 2010.

NDVI – Normalized Difference Vegetative Index which assesses the amount of live green material in the sampling area where 0 = no green material and 1 = ideal turf. Treatment number is equal to 1 = Control, 2 = 0.50% L, 3 = 1.00% 4 = 2.00% L, 5 = 4.00% L, 6 = 6.00% L, 7 = 8.00% L, 8 = 0.50% H, 9 = 1.00% H, 10 = 2.00% H, 11 = 4.00% H, 12 = 6.00% H, 13 = 8.00% H; where L (low surfactant) = 0.25% and H (high surfactant) = 1.00%. Southern Ag Surfactant for Herbicides was used as surfactant source. Different letters above treatment number bar indicate significant difference at the 0.05 level ($P<0.05$).

Root Measurements

No significant differences were found in root length among treatments for both years, revealing a lack of effect from fructose applications under the conditions experienced (Table 2.6). Date was found to be highly significant in terms of root length for 2009 (Table 2.6). The average decrease in root length across all treatments between the end of June and the end of July was 3.5 cm. Since the measurement from 2010 was of a destructive nature, root measurements could only be taken once, therefore date could not be analyzed.

Table 2.6 Average maximum root lengths by month for plots treated with Swanson 100% Pure Fructose during summer of 2009.

	Root Length[1]			
Treatment[2]	June	July	August	Mean[3]
	------------------------------- cm ---------------------------------			
Control	10.20 a	8.00 b	6.66 b	8.29
0.00% L	10.57 a	9.48 a	8.50 a	9.51
0.50% L	11.66 a	8.03 b	6.32 b	8.74
1.00% L	11.62 a	7.52 b	7.98 b	9.04
2.00% L	11.49 a	7.99 b	8.43 b	9.30
4.00% L	10.47 a	7.33 b	7.36 b	8.39
6.00% L	10.72 a	7.86 b	7.92 b	8.83
8.00% L	12.26 a	9.54 b	8.13 b	9.99
0.00% H	11.62 a	8.60 b	6.94 b	9.06
0.50% H	11.62 a	7.98 b	6.59 b	8.73
1.00% H	11.50 a	6.96 b	7.43 b	8.63
2.00% H	11.48 a	8.04 b	7.46 b	8.99
4.00% H	12.49 a	7.94 b	7.08 b	9.17
6.00% H	10.64 a	5.30 b	6.32 b	8.62
8.00% H	12.89 a	8.06 b	8.19 b	9.71
Mean[4]	11.41 a	8.17 b	7.42 c	

[1]Root length measurements were taken on 3 soil cores per plot and averaged within each treatment. Treatments are listed as percent sugar and rate of surfactant where L=0.25% and H=1.00%

[2]Treatment – Treatment column represents the level of sugar in the solution indicated by the percentage and the rate of surfactant indicated by H or L. Where H = 1.00% and L = 0.25%

[3]Mean corresponds to average root length within a treatment across all months.

[4]Mean corresponds to average root length within a month across all treatments. Different letters within a row indicate significant difference at the 0.05 level ($P<0.05$)

Clipping Measurements

Significant differences were found among treatments (Figure 2.11). Treatment 8 (0.50% H) proved to have the lowest clipping mass yield. However, there was no single treatment that clearly stood out as having the highest clipping mass. With the exception of treatment 8, all high surfactant treatments had a higher average mass than their low surfactant counterparts.

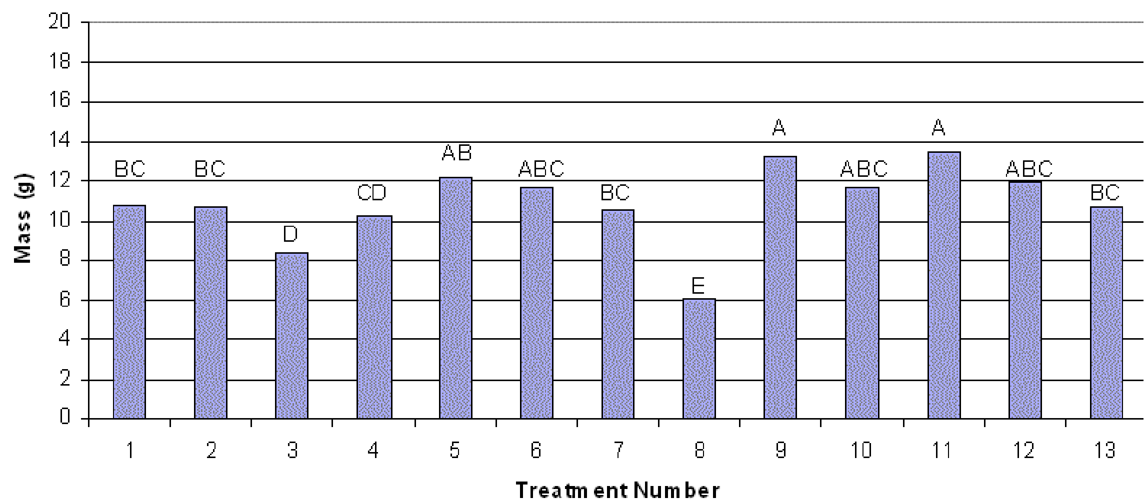

Figure 2.11 Clipping mass yield by treatment for pots treated with Swanson 100% Pure Fructose.

Treatment number is equal to 1 = Control, 2 = 0.50% L, 3 = 1.00% 4 = 2.00% L, 5 = 4.00% L, 6 = 6.00% L, 7 = 8.00% L, 8 = 0.50% H, 9 = 1.00% H, 10 = 2.00% H, 11 = 4.00% H, 12 = 6.00% H, 13 = 8.00% H; where L (low surfactant) = 0.25% and H (high surfactant) = 1.00%. Southern Ag Surfactant for Herbicides was used as surfactant source. Different letters above treatment number bar indicate significant difference at the 0.05 level (P<0.05).

Significant differences were found among weeks (Figure 2.12). Week 12 showed to have the lowest clipping mass yield. However, no week proved to have the highest clipping mass yield. Overall, a slight increase was experienced the first 5 weeks clipping data was recorded. However, the last two weeks showed significant decreases ending on its lowest level. A high clipping yield was not found to correspond with high turf quality. As clipping mass increased or decreased, turf quality typically did just the opposite.

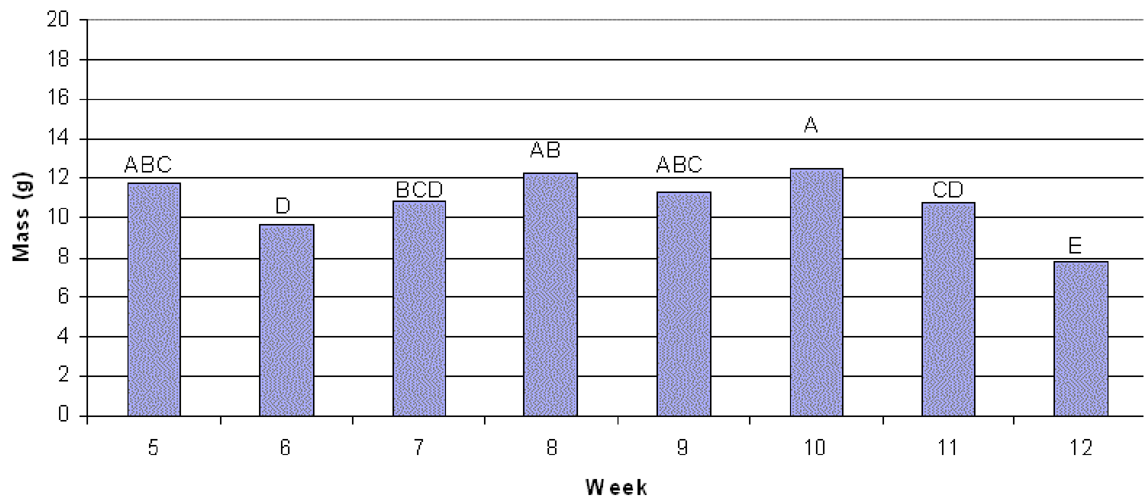

Figure 2.12 Clipping mass yield by week for pots treated with Swanson 100% Pure Fructose.

Different letters above treatment number bar indicate significant difference at the 0.05 level ($P<0.05$).

Table 2.7 Clipping mass yield[1] for 2010 across both fructose sources in regards to week.

WAT[2]	Cargill	Swanson	Mean[5]
	----------------- Mass (g)[3]	------------------	
5	12.13 b	11.74 abc	11.94 ab
6	13.05 b*	9.67 d	11.36 bc
7	15.33 a*	10.92 bcd	13.13 a
8	12.49 b	12.28 ab	12.38 ab
9	13.38 b	11.33 abc	12.36 ab
10	10.26 c	12.46 a*	11.36 bc
11	9.67 c	10.79 cd	10.23 c
12	7.85 d	7.74 e	7.79 d
Mean[4]	11.77 *	10.87	

[1]Clipping mass Yield is a measurement of dry material taken from the total weight of clippings.
[2]Week after initial treatment.
[3]Mass consists of grams/35.5 cm^2 pot.
[4]Means corresponds to average mass of clippings within a source across all treatments. Asterisk within a row indicates source was significantly higher at 0.05 level of significance ($P<0.05$).
[5]Mean corresponds to average mass of clippings within a week across both sources. Different letters within a column indicate significance at the 0.05 level ($P<0.05$).

Table 2.8 Clipping mass yield[1] for 2010 for both fructose sources in regards to treatment

Treatment[2]	Cargill	Swanson	Mean[5]
	--------------------------- Mass (g)[3]	---------------------------	
Control	15.54* ab	10.75 bc	13.15 a
0.50% L	15.33* ab	10.67 bc	13.00 a
1.00% L	13.00* c	8.38 d	10.69 bc
2.00% L	17.38* a	10.21 cd	13.79 a
4.00% L	13.79 bc	12.21 ab	13.00 a
6.00% L	12.63 cd	11.67 abc	12.15 ab
8.00% L	10.63 de	10.50 bc	10.56 bc
0.50% H	10.25* ef	6.08 e	8.17 d
1.00% H	7.54 g	13.21* a	10.38 c
2.00% H	9.96 ef	11.67 abc	10.81 bc
4.00% H	8.83 efg	13.42* a	11.13 bc
6.00% H	9.92 ef	11.88 abc	10.90 bc
8.00% H	8.21 fg	10.67* bc	9.44 cd
Mean[4]	11.77*	10.87	

[1]Clipping mass yield is a measurement of the dry material recovered from the total weight of clippings.

[2]Treatment – Treatment column is representing the level of sugar in the solution indicated by the percentage and the rate of surfactant indicated by H or L. Where H = 1.00% and L = 0.25%

[3]Mass consists of oven-dried clipping mass.

[4]Means corresponds to average mass of clippings within a treatment across both sources. Asterisk within a row indicates source was significantly higher at 0.05 level of significance (P<0.05).

[5]Mean corresponds to average mass of clippings within a source across all treatments. Different letters within a column indicate significance at the 0.05 level (P<0.05).

DISCUSSION

Cargill Fructose Source

The effect of Cargill fructose on turf quality was fairly consistent between 2008 and 2010. For both years, fructose treatments with the high rate of surfactant displayed lower turf quality than those receiving the low rate of surfactant regardless of sugar level. Sorochan (2002) also found fructose treatments to yield unacceptable turfgrass injury unless applied with 0.1% rate of surfactant. No treatments from the field study were significantly better than the control, whereas two were better in the glasshouse study but did not show up in NDVI readings. Sorochan (2002) found turfgrass treated with 1.25% rate of fructose to be the ideal rate at preventing turfgrass injury. This study also found low rates to be the only not to cause turfgrass injury.

In both years, no treatment was significantly higher than the control in relation to NDVI. Both years had a distinct difference in NDVI levels between plots receiving the low level of surfactant and plots receiving the high level. Both years had some treatments with fructose rates less than or equal to 2.00% that had higher averages than the control. Both years showed that the three highest rates of fructose coupled with high surfactant resulted in the lowest overall NDVI average.

The overall decrease in turf quality and NDVI was a result of prolonged high temperatures (29-35˚C) occurring throughout the summer months, coupled with reduced irrigation. This led to the cool-season turf showing signs of stress. The cumulative effect of high temperatures leads to each stress situation becoming more severe to the plant. If the Cargill fructose was providing any benefit to visual ratings associated with the initial weeks of the study, it appears to be short lived. As fructose was consistently applied over the course of the study, it may have had a residual effect eventually leading to a decrease in turf quality. The increased sticky nature of the syrup could have the effect of staying on the leaves of the plant without entering them. The residual high fructose corn syrup would continue drawing water out of the plant, thus the decrease in quality. This coincides with clipping measurements results in which there was a temporary increase followed by a steady decrease as fructose levels increased in the turf. Sorochan (2002) found turfgrass injury to increase, regardless of fructose level, when applied 5 times per week to supina bluegrass under reduced light conditions. Juhren and Went (1949) found plants treated with sucrose and grown in the greenhouse under high light intensities had severely reduced growth when compared with controls.

Swanson Fructose Source

The lack of a pattern in regards to turf quality and NDVI was experienced for both years, confirming that the fructose does not appear to be having an effect. Went and Carter (1948) found a 10% sucrose solution sprayed in daylight under controlled temperatures to result in less than or equal to growth rates of tomato plants when compared to control plants. This also showed a lack of effect from sugar applications. The differences could be attributed to original health of the plants in the pot, which did not show up initially but came to fruition over the course of the study. Higher clipping mass did not correspond to higher turf quality or NDVI, as those measurements found no increases as fructose levels increased. The applied fructose could be adding to the mass of the plant without improving any quality measurements.

No significant differences were found in root measurements between treatments. This coincides with findings from Amiard et al., (2003) in which only 0.9% of applied fructose to *Lolium perenne* L. was found allocated to the roots. Also, Went and Carter (1947) found sucrose applied to the roots of tomato plants to be negligible in terms of growth. Rankings in average root length appear to have no real pattern, coinciding with visual ratings and NDVI readings.

The significant differences found in date over both years is hard to associate with fructose treatments due to the random pattern. This may indicate that the fructose itself is not causing the differences but rather some other factor such as heat, humidity, or care. High temperature stress is quite common in CBG, so it is not unusual to see a decline in overall health. In field situations injury patterns from summer stress can be random (Dernoeden, 2002). No disease symptoms or signs were experienced during the study, so a decline resulting from fungi is not likely. The significant decline in root measurements over the course of the 2009 study can be a result of soil heating that leads to death of roots (Dernoeden, 2002). This significant decrease is a substantial loss of root material and greatly affects the plant's ability to survive summer heat. This root loss could be responsible for the loss of turf quality over the course of the summer, indicating the fructose failed to offset negative effects of summer stress. With the decrease in root length, this leads to the turf's reduced ability to absorb water and nutrients from the soil (Dernoeden, 2002). With decreased water uptake, stress levels are increased leading to a greater propensity for wilting (Dernoeden, 2002) and a reduction in turf quality. This coincides with typical CBG heat stress in which high temperatures cause root hairs to die, roots to turn brown, and eventual loss of proper function (Dernoeden, 2002). A reduction in photosynthates due to photorespiration results in inadequate energy produced during respiration to maintain a sufficient root system (Dernoeden, 2002). Stressed plants are then more subject to pests such as insects, weeds, and diseases (Dernoeden, 2002; Turgeon, 2008).

Both Fructose Sources

Even with inconclusive results from the Cargill study, some patterns could be deduced such as high levels of sugar and surfactant having a negative effect on turf quality. However, Swanson results were also inconsistent in determining a positive effect on turf quality. Temperatures in the field between 2008 and 2009 were similar; however, the 2009 study period received 36.65 cm of rain while there was only 16.43cm in 2008. This may account for some of the overall higher quality of 2009.

Data from Cargill source had much more variability, with the range in average turf quality being nearly two points. For NDVI only 0.028 units for 2009 and 0.04988 for 2010 on a 0-1 scale separated the highest average from the lowest average in regards to treatment. When contrasting with the Cargill fructose source, it experienced differences overall of nearly 0.1 units. The larger range of NDVI from the Cargill source was from lower end average scores while both sources experienced similar ratings on the upper end. Even though Swanson had 52% greater roots in the 2010 study, other data suggests that neither product was helping to improve turf quality or NDVI; rather the Swanson source did less to harm to the turf. This coincides with Swanson having a greater NDVI than its Cargill counterparts. Once again, this points to the high fructose corn syrup having a longer adherence to leaf blades and causing harm to the plant at the higher rates. Went and Carter (1948) found a 10% sucrose solution sprayed in daylight under controlled temperatures to result in less than or equal to growth rates of tomato plants when compared to control plants. This also showed a lack of effect from sugar applications.

A significant difference was found between sources with Swanson resulting in 52% greater root than Cargill treated plants. This coincides with Swanson having a greater NDVI than its Cargill counterparts and may indicate that the Swanson product was either less deleterious to the turf, or may actually be slightly beneficial to root growth.

When clipping mass yield is averaged across both sources, significant differences are found (Table 2.7, 2.8). The Cargill source had a significantly higher mass when compared with pots treated with the Swanson source. This mass does not correspond to higher turf quality, since NDVI readings found the Swanson source to produce a higher average overall. As expressed earlier it appeared the Cargill source had a residual effect on mass without positively effecting turf quality.

Since there appears to be a lack of effect due to fructose applications for the Swanson study, it is possible we were unsuccessful in getting the fructose into the plant.

As was discovered during a later surfactant screen (Chapter 3), the hydrophilic to lipophillic balance (HLB) of the surfactant used for this portion was not appropriate for obtaining maximum absorption. Without the fructose entering the plant it cannot be used to offset photorespiration. The miscible Swanson product would then be washed into soil matrix where it would quickly be consumed by soil microbes. The high fructose corn syrup in the Cargill source also contained other compounds in addition to the fructose. These compounds along with it being syrup-based may have caused the product to 'stick' to the leaves longer while not being absorbed due to improper surfactant. This adherence to the leaves could have actually drawn moisture out of the plant, much like an excessive nitrogen fertilizer application (Turgeon, 2008) resulting in the reduced turf quality experienced from high rates in 2008. Sorochan (2002) found rates above 1.25% of high fructose corn syrup to result in unacceptable turfgrass injury when applied 5 times per week to supina bluegrass.

CONCLUSIONS

Fructose applied by either Cargill 42 High Fructose Corn Syrup or Swanson 100% Pure Fructose showed no benefit to visual ratings or NDVI measurements. High surfactant rates with the Cargill source resulted in turfgrass injury regardless of fructose rate. Also, low surfactant with high fructose rates resulted in poor turf quality. Turf treated with the Swanson source failed to show to a patterned treatment effect. While it did not result in significant injury like its Cargill counterpart, it also failed to show noticeable improvement on a consistent basis. If either of these sources is providing some benefit at alleviating photorespiration, it was failed to be seen during the experiment timeframe on the parameters tested. Turf treated with Cargill showed increased clipping yield when compared to Swanson, but this did not translate to improved turf quality or total root length. Cargill had significantly less root length than turf treated with Swanson. Further research could be warranted by looking into using different surfactant sources and less frequent fructose applications.

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CHAPTER III

EFFECT OF A SURFACTANT'S HYDROPHILLIC TO LIPOPHILLIC BALANCE ON FRUCTOSE ABSORPTION ON CREEPING BENTGRASS

ABSTRACT

Creeping bentgrass subjected to heat-stress undergoes a negative process known as photorespiration in which it fails to produce sugar from photosynthesis. Turf managers are faced with increased labor practices in order to keep the turf alive. Applying sugar in the form of fructose is a possible way at alleviating the negative effects of photorespiration. In hopes of achieving maximum foliar absorption a surfactant was necessary. However, due to lack of past research, the type and rate to maximize absorption was unknown. One aspect in particular was the surfactants hydrophilic to lipophillic balance (HLB). The objective of this study was to determine which HLB of a surfactant and which rate of a surfactant maximized absorption of fructose. Rate and HLB had a significant impact on absorption. As HLB increased with a soluble source so did absorption. With fructose, the greatest absorption was found with HLB=18.3, the highest available HLB. Absorption was also found to increase as rate of surfactant increased to a point between 0.25% and 1.00%. The 0.25% rate had the highest results while 1.00% had the least absorption.

INTRODUCTION

Creeping bentgrass (CBG) (*Agrostis stolonifera* L.) is a fine textured, cool-season turfgrass. It has been used on everything from home lawns to golf courses. However, its dense canopy, fine texture, spreading stolons, and low mowing tolerance, make it the most popular and highest quality choice for golf course fairways and putting greens (Christians, 1998; Beard 2002; Koh et al., 2003; Turgeon, 2008; Cooper and Peacock 2008). The popularity and publicity of golf has associated CBG putting greens with premier facilities. This demand has resulted in it being spread to areas not adapted for its optimal growth (Christians, 1998). When grown in these areas, the turf must receive constant care during the summer months. While CBG does present an excellent putting surface, the amount of labor, chemicals, and irrigation required to keep this surface alive south of the transition zone is insufficient during some years (Dernoeden, 2002).

When CBG is subjected to extreme stress, it is unable to carry out photosynthesis to its full potential. When C_3 plants are subjected to high temperatures, the plant begins a process known as photorespiration. This efficiency robbing pathway binds O_2 rather than CO_2 at high temperatures, allowing only one molecule of 3-phosphoglyceric acid (3 PGA), the precursor to glucose, to be formed rather than two. As a result, no sugar can be generated and CO_2 is released back into the atmosphere (Turgeon, 2008). Without the main sugar being produced from photosynthesis, respiration levels decrease resulting in slower leaf and root growth from lack of energy.

As the plant is conducting photosynthesis, the production of carbohydrates usually exceeds the use of carbohydrates (Waddington et al., 1992). Cool-season grasses tend to store carbohydrates as long chain polymers of fructose molecules, or fructans (Waddington et al., 1992). However, due to photorespiration, plants do not make sugars and thus have reduced energy stores of fructans to utilize when growing conditions become unfavorable and the plant is unable to conduct photosynthesis.

Surfactants (surface acting agents) are used in a wide variety of situations and are a type of adjuvant. Adjuvants are any material added to a spray solution to increase its performance (Hess, 1999) Penner and Ronggenbuck (1999) found an adjuvant was necessary for fructose to be absorbed by the plant. A common misconception is to assume all adjuvants and furthermore surfactants, to be the same (Penner, 2000). It is essential to match the surfactant appropriately to the spray solution based on the solution's characteristics and the target's characteristics (Penner, 2000). One such characteristic is the surfactant's hydrophilic to lipophillic balance (HLB). The HLB value is usually given on a scale of 1-20, with lower numbers being for more lipophillic solutions and higher numbers recommended for more hydrophilic solutions (Hess, 1999). It has been shown that the HLB value of a surfactant applied with an herbicide has had a great effect on the amount of uptake and control (Green and Green, 1993; Nalewaja et al., 1996a; Manthey et al., 1996a; Manthey et al., 1996b; Nalewaja et al., 1996b; Nalewaja et al., 2001). As water solubility of the compound increases, the HLB of the surfactant should also increase to maximize absorption (Stock and Holoway, 1993).

If applied fructose were to reach the soil, it would most likely be broken down by soil microbes before root absorption could occur. Therefore, it is imperative absorption occur through openings in the leaf blades. A surfactant is needed to increase absorption chances. Due to fructose's high solubility it was hypothesized that a surfactant with a high HLB would have the best absorption. The objectives of this screen were to test surfactants with varying HLB values and rates, to see which HLB, rate, and combination had the greatest absorption rate of fructose into CBG leaves. The information generated could therefore be used in subsequent studies.

MATERIALS AND METHODS

A series of surfactants, Dow Triton X-Series (The Dow Chemical Company, Midland, MI), was obtained since there are no recommendations on the type of surfactant to use with fructose applications. The surfactants included Triton X-15, Triton X-35, Triton X-135, Triton X-705 and were selected based on the range of hydrophilic to lipophillic balance (HLB) values they provided (4.8, 7.8, 15.5, 18.3, respectively). Clippings were obtained from 'A4' CBG pots being grown in a glasshouse. The clippings were weighed into 0.5g aliquots and placed in a centrifuge tube. A 50 mL flask was filled with 40 mL of deionized water. The water was brought to a 1% fructose solution by adding 0.4g of ^{12}C fructose and 20 µL of ^{14}C fructose. Surfactant was then added to make a 0%, 0.1%, 0.25%, or 1.0% surfactant solution. The solution was then placed on the stirring plate for 5 minutes. Ten milliliters of the 1% fructose solutions was added to each centrifuge tube containing 0.5g of clippings. For each rate of surfactant, this was replicated 3 times, leaving 10 mL of solution as a disintegrations per minute (DPM) control. Centrifuge tubes were placed on the tumbler and allowed to tumble for 1 hour. Tubes were then placed in the centrifuge and spun for 5 minutes at 2000 rpm. Three 1 mL subsamples were taken from each centrifuge tube. Each 1 mL aliquot was mixed with 10 mL of Aquasol-2 cocktail (Perkin Elmer, Waltham, MA) in liquid scintillation vials. Vials were then placed in the LSC to measure DPM. The experiment was then repeated 4 times. Data were analyzed using the general linear model procedure of the Statistical Analysis Software (SAS Institute 9.2, Cary, NC). Mean separation was conducted using Fisher's protected LSD with an alpha level of 0.05.

Results were recorded to determine the best overall HLB for absorption, the best rate of surfactant for absorption, and the best combination of HLB and rate of surfactant for absorption.

RESULTS AND DISCUSSION

No significant interaction was found between rate and HLB. Significant differences were found between surfactants of varying HLB values across all rates of surfactant. It was found as HLB increased so did the percent absorption of the fructose in a linear manner (Figure 3.1). The surfactant with HLB of 18.4 had the highest absorption rate of 2.87%. Samples treated with surfactant with HLB of 4.9 had the significantly lowest average absorption. Data suggests that regardless of the rate of surfactant, a higher HLB value should be used to maximize absorption of fructose due to its high solubility. This finding is in agreement with previous studies that found the HLB value of a surfactant applied with a herbicide has a great effect on the amount of absorption and control (Green and Green, 1993; Nalewaja et al., 1996a; Manthey et al., 1996a; Manthey et al., 1996b; Nalewaja et al., 1996b; Nalewaja et al., 2001) Also, as water solubility of the compound increases, the HLB of the surfactant should also increase to maximize absorption (Stock and Holoway, 1993).

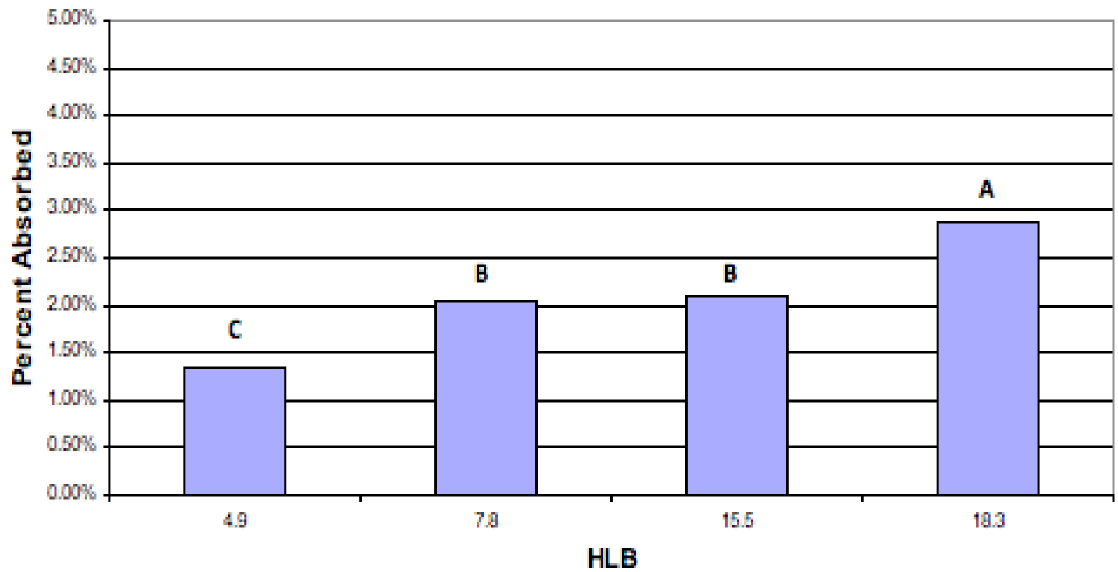

Figure 3.1 Percent ^{14}C fructose absorbed across all rates of surfactant.

Different letters above percent absorption bar indicate significant difference at the 0.05 level (P<0.05).
*HLB – Hydrophilic to Lipophillic Balance.

In order to determine the ideal rate and HLB of the surfactants, results were broken down into individual rates of surfactant and absorption was observed by HLB value. Samples that received no surfactant were still grouped by HLB value during testing. As was expected, the zero surfactant treatment showed very little differences on absorption between samples (Table 3.1). This was expected since all treatments were essentially the same, thus reinforcing our confidence the chosen method could produce reliable results.

Table 3.1 Percent ^{14}C fructose absorbed across various surfactant rate and HLB values.

	Surfactant Rate				
HLB[1]	0.00%	0.10%	0.25%	1.00%	Mean[4]
	------------------------ Percent Absorbed (%) ------------------------				
4.9	1.28	1.66 b	1.52 b	0.87b	1.33 c
7.8	1.60 B	3.02 Aab	1.89 ABb	1.69 Bab	2.05 b
15.5	2.06 AB	1.90 ABab	2.79 Ab	1.59 Bab	2.09 b
18.4	1.88 B	3.28 ABa	4.35 Aa	1.98 Ba	2.87 a
Mean[3]	1.70 B	2.46 A	2.72 A	1.53 B	

[1]HLB – Hydrophilic to Lipophillic Balance
[2]Surfactant Rate represents amount of surfactant in a given amount of solution.
[3]Mean corresponds to average percent absorbed within a surfactant rate across all HLB values. Different capital letters within a row indicate a significant difference at the 0.05 level ($P<0.05$).
[4]Mean corresponds to average percent absorbed with a HLB value across all surfactant rates. Different lower-case letters within a column indicate a significant difference at the 0.05 level ($P<0.05$).

Significant differences were found on fructose absorption between HLB values and a 0.1% surfactant level (Table 3.1). With the exception of HLB 15.5, as HLB increased so did mean absorption rate. The HLB 18.4 had the highest mean % absorption. All treatments had a higher mean absorption than their 0.00% rate counterpart. Again, with the exception of HLB 15.5, results for this surfactant rate (0.1%) held true to our theory that increasing HLB will increase absorption of ^{14}C fructose.

Samples receiving the 0.25% level of surfactant had significant differences between HLB values. As theorized, as HLB increased so did mean percent absorption amount (Figure 3.2). Samples receiving surfactant with HLB 18.4 had the highest percent absorption at 4.35%. The remaining three surfactants did not show statistical difference, however the mean increased as HLB value increased. All treatments had higher absorption rates than their 0.00% counterparts, reinforcing the benefit of surfactant to absorption.

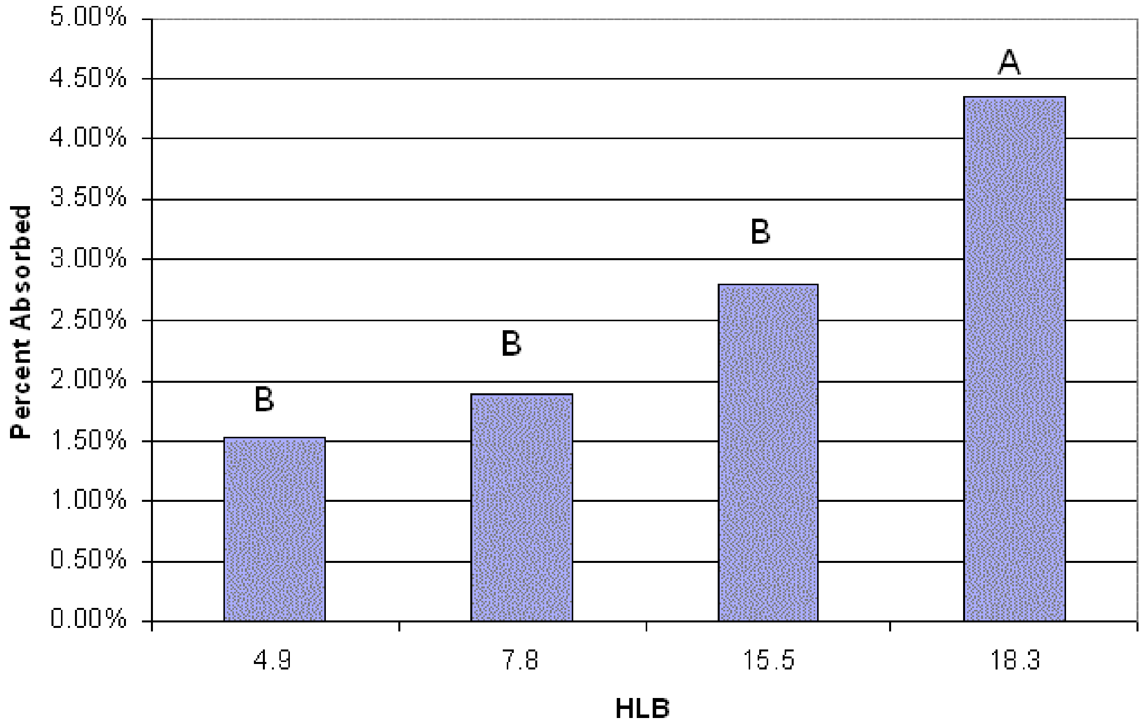

Figure 3.2 Percent ^{14}C fructose absorbed across all 0.25% of surfactant.

Different letters above percent absorption bar indicate significant difference at the 0.05 level (P<0.05). HLB refers to Hydrophilic to Lipophillic Balance

Samples receiving the 1.00% level of surfactant resulted in significant differences between HLB values. As with the other two levels of surfactants, the overall trend of increasing absorption with increasing HLB was observed here, however, the differences were not as great, nor was the percent absorbed (Table 3.1). Also, the absorption values at this rate and of those at the 0.00% rate are very similar. The lack of uptake could be a result of there being too much surfactant. Green and Brown (1990) found a surfactant rate of 1.00% to reduce sulfonylurea activity in corn and cause damage to the plant. Sorochan (2002) found a high rate of surfactant (0.25%) caused unacceptable turf quality regardless of rate of fructose applied to supina bluegrass (*Poa supina*). Perhaps the high concentration may be helping the fructose attach to the leaf surface, but by being at such high concentrations it could clog the stomates and result in no fructose entering the plant.

It was hypothesized that the addition of a surfactant would increase the rate of absorption of fructose into the leaves of CBG. It was also hypothesized that as rate of surfactant increased so would percent absorption to a certain point, with too much surfactant actually hindering absorption. This was found to be the case by Green and Green (1993) with surfactant increasing rimsulfuron activity 10 fold at 0.1% over the control. Significant differences were found in regards to surfactant rate across all HLB values (Figure 3.3). It was found as rate of surfactant increased from 0.0% to 0.25% so did the rate of absorption. As the rate increased from 0.25% to 1.0% surfactant, the rate of absorption significantly decreased. No surfactant rate resulted in consistently higher absorption than others. The 0.25% rate had the highest overall mean percent absorption at 2.72%. The 0.1% rate had the second highest absorption rate at 2.46% but was not significantly different than the 0.25% rate. Samples that received no surfactant had the third highest rate of absorption at 1.70%. The amount was significantly lower than the 0.25% rate, but had no significant difference from the 0.1% rate. The 1.0% rate of surfactant had the lowest percent absorption overall at 1.53%. This amount was significantly lower than the 0.25% and 0.1% rates but had no significant difference from the samples that were untreated. This data adheres to our hypothesis and indicates that between a surfactant rate of 0.25% and 1.0%, the surfactant actually begins to impede absorption. One possibility is the large amount of surfactant impedes absorption by clogging the pores on the leaf surface.

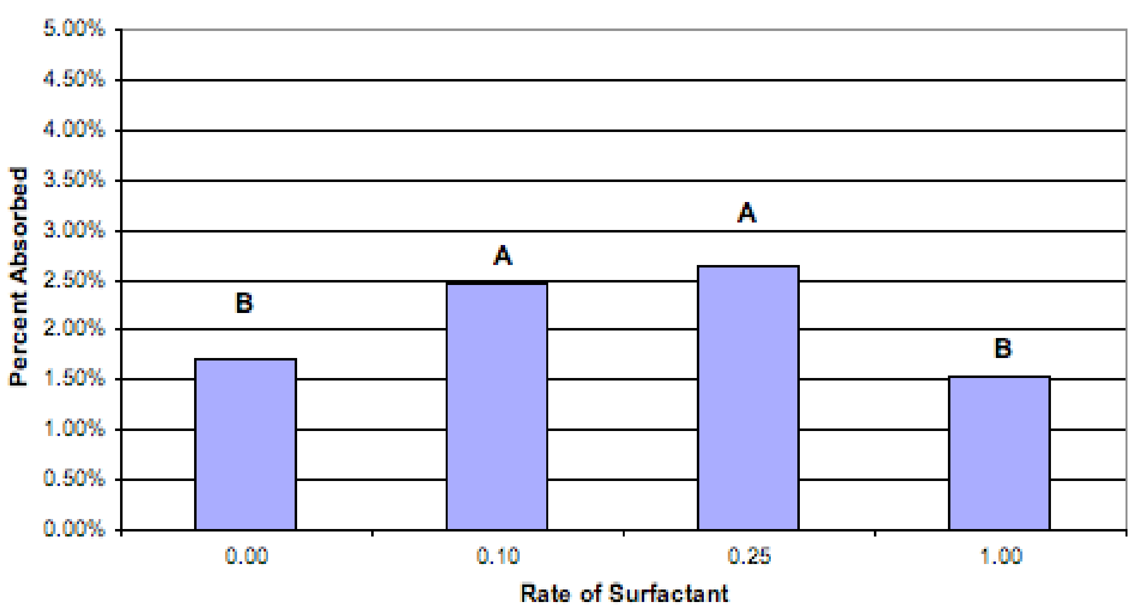

Figure 3.3 Percent ^{14}C fructose absorbed across all HLB values by rate of surfactant. Different letters above percent absorption bar indicate significant difference at the 0.05 level (P<0.05).

When the varying surfactants were broken down by HLB value, it was found that 3 of the 4 surfactants had significant differences between rates. The HLB 4.9 did not have significant differences between rates of surfactant (Table 3.1). These results were expected since HLB value has a significant impact on the amount of absorption. Surfactants with low HLB values fail to aid in absorption of hydrophilic compounds (Hess, 1999; Hazen, 2000). Using a surfactant with an HLB value of 4.9 will have no significant effect on absorption of fructose regardless of the rate of surfactant.

Samples that received surfactant with HLB 7.8 had significant differences between rates of surfactant (Table 3.1). No rate proved to be the consistently advantageous. Samples receiving a rate of 0.1% had the highest average absorption at 3.02%. Samples receiving the 0.25% rate had the second highest average absorption at 2.21%, but were not statistically different from the 0.1% rate. Samples getting the 1.0% rate averaged 1.67% and samples receiving no surfactant averaged 1.60% absorption. These amounts were significantly lower than the 0.1% rate but not different from the 0.25% rate.

Samples that received surfactant with HLB 15.5 showed significant differences between rates of surfactant (Table 3.1). Once again no rate proved to be the consistent best. The 0.25% rate had the highest overall average at 2.79% absorption. Untreated samples had the second highest average at 2.06%, but was not statistically different from the 0.25% rate. Samples getting the 0.1% rate and 1.0% rate had absorption amounts of 1.90% and 1.59%, respectively.

Samples that received surfactant with HLB 18.3 experienced significant differences between rates of surfactant (Table 3.1). Once again no rate proved to be the overall best. Samples receiving the 0.25% rate had the highest average percent absorption for this HLB value and all other HLB values at 4.35%. The 0.1% rate had a lower average at 3.27% absorption, but was not statistically different than the 0.25% rate. The 1.0% and 0.00% rates had very similar averages at 1.98% and 1.88% respectively. These were statistically worse than the 0.25% rate, but not the 0.1% rate. The HLB 18.3 treatments had the highest percent absorption average for an individual rate and the highest overall percent absorption average.

This set of experiments showed that a surfactant with a high HLB and a rate similar to 0.25% would give the best absorption when used with fructose. This information would have been useful when selecting materials for the field studies. Without a prior knowledge of HLB values, this characteristic was not considered when selecting surfactants. Since this became apparent, the surfactants used in the field studies had a HLB value in the 10-12 range, which is common for turf surfactants since they are commonly used for a range of applications. By having a median HLB value this allows use for all types of herbicides. Whereas a surfactant with an HLB on one end of the spectrum would have a very limited application use. Had a surfactant with a higher HLB been used, results may have been different. If the surfactant used failed to get adequate fructose in the plant then this could explain the lack of visible results. It could also explain the negative effects experienced from the Cargill fructose source. The fructose with the 1.00% rate of surfactant all experienced a negative impact on turf quality and NDVI, which coincides with our surfactant screen results. A 1.00% rate was also found to reduce sulfonylurea activity and caused damage to the plant (Green and Brown, 1990). This reduction may have resulted from the fructose failing to enter the plant and creating a diffusion gradient in which water was pulled out of the plant. If lab results translated to the field, then perhaps more fructose would have entered the plant if a higher HLB had been used, providing different results.

CONCLUSIONS

The HLB of the surfactant used with the fructose has a significant impact on the amount of absorption. Rate of surfactant also plays a significant role in absorption. As HLB increases with a soluble source so does absorption. With fructose, the greatest absorption was found with HLB=18.3, the highest available HLB. Absorption was also found to increase as rate of surfactant increased to a point. The 0.25% rate had the highest results, with the next rate, 1.00% having the least absorption. This study was successful at revealing the impact HLB and surfactant rate play on absorption of fructose. Further research could be warranted at evaluating more surfactants at more rates to determine to exact point in which surfactant starts to have a negative impact on absorption. Further, these results could be translated to field application of fructose to heat stressed creeping bentgrass with the objective of increasing absorption and reducing negative effects of photorespiration.

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APPENDIX

DATA FROM FIELD, LAB, AND GLASSHOUSE STUDIES

Table A.1 Main effects of fructose and surfactants treatments on visual rating of turf quality for summer of 2008.

	Date												
	17-Jul	24-Jul	1-Aug	7-Aug	15-Aug	21-Aug	29-Aug	5-Sep	12-Sep	19-Sep	22-Sep	2-Oct	Total
	-----1-9 Scale-----												
Treatment	--------------												
Control	7.00	7.25	7.00	7.25	8.00	7.25	7.75	7.25	7.25	7.50	7.00	7.50	7.31 ab
0.00% L	7.00	7.67	7.33	7.67	8.00	7.00	7.67	7.00	7.33	7.00	6.67	7.33	7.31 ab
0.25% L	7.50	8.00	8.00	7.67	8.00	7.33	8.00	7.00	7.00	7.00	7.00	7.33	7.50 a
0.50% L	7.50	7.67	7.67	7.67	7.67	7.33	8.00	7.00	7.00	7.33	7.00	8.00	7.47 a
0.75% L	7.33	6.67	7.00	7.33	7.33	7.33	8.00	7.33	7.33	7.33	7.33	7.33	7.22 ab
1.00% L	7.67	7.00	7.00	7.67	7.33	7.00	7.33	7.00	7.00	7.00	7.00	7.33	7.14 bc
1.50% L	7.50	7.00	7.67	7.67	7.67	7.33	7.33	7.33	7.33	7.33	7.00	7.33	7.36 ab
2.00% L	7.50	7.00	7.33	6.67	7.33	7.33	8.00	7.00	7.00	7.67	7.00	8.00	7.25 ab
4.00% L	7.33	7.00	6.67	6.67	7.00	7.00	8.00	6.67	6.33	7.00	6.67	7.67	6.92 cd
6.00% L	7.67	6.67	6.67	6.67	7.00	7.00	7.00	6.00	5.33	6.00	6.33	6.33	6.50 ef
8.00% L	7.50	6.00	6.00	6.00	6.67	7.00	7.00	6.67	6.00	6.33	6.33	7.00	6.47 ef
0.00% H	7.50	7.67	6.67	6.67	7.00	6.00	7.00	5.67	7.00	6.67	6.67	7.00	6.75 de
0.25% H	6.67	7.00	7.00	7.00	7.00	6.33	8.00	6.33	6.33	6.67	7.00	7.33	6.92 cd
0.50% H	7.33	7.33	7.33	7.33	7.00	6.00	7.67	6.67	6.67	7.00	7.00	7.67	7.08 bc
0.75% H	7.00	6.67	7.00	6.67	7.00	6.33	7.00	6.33	6.67	6.67	6.33	6.67	6.64 def
1.00% H	7.00	7.67	6.67	6.67	7.00	6.00	6.67	6.00	6.00	5.67	6.33	7.00	6.58 ef
1.50% H	7.00	7.00	6.67	6.67	6.67	6.00	6.33	6.33	6.67	6.67	6.67	7.33	6.64 def
2.00% H	7.33	6.67	6.67	7.00	7.00	5.67	6.00	6.00	6.00	6.00	6.00	7.00	6.39 f
4.00% H	7.00	6.67	6.00	6.33	6.33	5.67	6.33	5.33	5.67	5.67	5.33	6.33	6.03 g
6.00% H	7.00	5.67	5.00	6.33	6.00	6.00	6.67	5.33	5.00	6.33	5.33	6.67	5.81 g
8.00% H	7.67	4.67	5.00	5.67	5.33	5.67	6.00	4.67	4.33	4.67	5.67	6.33	5.06 h
LSD	1.09	0.81	0.66	0.80	0.73	0.69	0.80	0.98	0.88	1.06	1.02	0.84	0.29

[1]Turf Quality was rated visually on a 1 to 9 scale, 1 = brown, dead turf, 7 was the minimum value for acceptable golf course putting green turf quality, 9 = ideal turf.

Table A.2 Main effects of fructose and surfactants treatments on NDVI readings during summer 2008.

	Date										
	24-Jul	1-Aug	7-Aug	15-Aug	21-Aug	29-Aug	5-Sep	12-Sep	22-Sep	6-Oct	Average
Treatment.[1]	NDVI[2] 0 – 1 Scale										
Control	0.747	0.770	0.738	0.758	0.779	0.840	0.781	0.736	0.683	0.782	0.761 abc
0.00% L	0.767	0.783	0.752	0.762	0.766	0.835	0.781	0.725	0.673	0.767	0.761 abc
0.25% L	0.770	0.773	0.754	0.755	0.763	0.837	0.773	0.730	0.690	0.775	0.762 abc
0.50% L	0.767	0.778	0.751	0.759	0.777	0.844	0.785	0.736	0.703	0.768	0.767 a
0.75% L	0.740	0.766	0.722	0.745	0.762	0.843	0.781	0.736	0.690	0.759	0.754 bcde
1.00% L	0.757	0.781	0.739	0.759	0.758	0.826	0.760	0.709	0.684	0.766	0.754 cde
1.50% L	0.757	0.771	0.736	0.748	0.766	0.842	0.783	0.728	0.695	0.771	0.760 abc
2.00% L	0.758	0.769	0.744	0.761	0.778	0.845	0.778	0.740	0.707	0.776	0.765 ab
4.00% L	0.735	0.750	0.720	0.738	0.749	0.816	0.755	0.691	0.643	0.766	0.736 fg
6.00% L	0.705	0.757	0.687	0.717	0.728	0.800	0.722	0.639	0.606	0.746	0.711 hi
8.00% L	0.695	0.709	0.677	0.709	0.743	0.824	0.752	0.652	0.653	0.745	0.716 h
0.00% H	0.766	0.764	0.734	0.734	0.758	0.836	0.780	0.737	0.715	0.766	0.759 abcd
0.25% H	0.742	0.752	0.728	0.742	0.752	0.833	0.772	0.717	0.683	0.760	0.748 de
0.50% H	0.753	0.765	0.729	0.733	0.735	0.822	0.770	0.718	0.674	0.781	0.748 de
0.75% H	0.738	0.757	0.710	0.719	0.737	0.820	0.762	0.699	0.672	0.738	0.735 fg
1.00% H	0.752	0.758	0.715	0.724	0.732	0.818	0.752	0.688	0.644	0.732	0.732 g
1.50% H	0.744	0.757	0.721	0.722	0.732	0.819	0.780	0.718	0.693	0.755	0.744 ef
2.00% H	0.746	0.767	0.712	0.717	0.714	0.802	0.744	0.670	0.672	0.747	0.729 g
4.00% H	0.728	0.741	0.698	0.700	0.706	0.794	0.724	0.636	0.641	0.730	0.710 hi
6.00% H	0.694	0.703	0.679	0.683	0.715	0.798	0.736	0.638	0.641	0.728	0.702 i
8.00% H	0.674	0.689	0.658	0.664	0.694	0.771	0.686	0.575	0.578	0.709	0.670 j
LSD	0.025	0.019	0.019	0.020	0.020	0.019	0.031	0.044	0.056	0.040	0.011

[1]Treatment – Treatment column is representing the level of sugar in the solution indicated by the percentage and the rate of surfactant indicated by H or L. Where H = 1.00% and L = 0.25%

[2]NDVI – Normalized Difference Vegetative Index which assesses the amount of live green material in the sampling area where 0 = no green material and 1 = ideal turf.

Table A.3 Main effects of fructose and surfactants treatments on visual ratings of turf quality for summer of 2009.

	Turf Quality[1]												
	3-Jun	10-Jun	18-Jun	25-Jun	2-Jul	9-Jul	17-Jul	23-Jul	31-Jul	7-Aug	14-Aug	24-Aug	Total
Treatment	------ 1 – 9 Scale ------												
Control	7.00	7.00	7.00	7.00	7.00	7.00	6.33	6.67	6.67	6.33	7.00	7.33	6.86 cdefg
0.00% L	6.67	6.67	7.00	6.33	6.33	6.67	6.33	6.67	6.33	6.33	6.67	7.00	6.58 g
0.50% L	7.33	6.67	7.37	6.67	7.33	7.33	6.67	6.67	7.33	6.67	7.33	7.33	7.06 abcd
1.00% L	7.33	7.00	7.00	6.33	6.67	7.00	6.00	6.33	6.67	6.33	7.00	7.67	6.78 efg
2.00% L	7.33	7.00	7.37	6.67	7.00	7.33	6.67	7.00	7.00	7.00	7.00	7.67	7.09 abc
4.00% L	6.67	6.67	7.33	6.67	7.33	7.00	6.33	7.00	6.67	6.67	6.67	7.00	6.83 cdefg
6.00% L	7.33	7.33	7.67	6.67	6.67	6.67	6.33	6.00	6.33	6.33	6.67	6.67	6.72 fg
8.00% L	7.33	7.67	8.00	7.33	7.33	7.33	7.00	7.00	7.00	7.00	7.00	7.33	7.28 ab
0.00% H	7.67	7.67	7.67	7.33	7.33	7.67	6.67	6.67	7.00	7.00	7.67	7.33	7.31 a
0.50% H	7.00	7.67	7.33	7.33	6.67	7.00	5.67	5.67	6.67	5.33	6.67	7.00	6.67 g
1.00% H	7.00	7.00	7.33	7.00	7.00	6.67	5.67	6.67	6.33	6.00	7.00	7.33	6.75 fg
2.00% H	6.67	7.33	7.33	7.00	7.00	7.33	6.33	7.00	6.67	7.00	7.00	8.00	7.06 abcde
4.00% H	7.00	7.33	7.33	7.00	6.67	7.33	6.00	6.00	6.67	6.33	7.00	7.00	6.81 defg
6.00% H	7.00	7.00	7.33	7.00	6.67	7.00	5.33	5.67	6.33	5.67	7.00	7.00	6.58 g
8.00% H	7.00	7.00	7.67	7.33	7.33	7.33	6.33	6.67	7.00	6.33	6.67	7.33	7.00 bcdef
LSD	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.289

[1]Turf Quality was rated visually on a 1 to 9 scale, 1 = brown, dead turf, 7 was the minimum value for acceptable golf course putting green turf quality, 9 = ideal turf.

Table A.4 Main effects of fructose and surfactants treatments on NDVI readings during summer 2009.

	NDVI[2]												
	3-Jun	10-Jun	18-Jun	25-Jun	2-Jul	9-Jul	17-Jul	23-Jul	31-Jul	7-Aug	14-Aug	24-Aug	Average
Treatment[1]	---------- 0 – 1 Scale ----------												
Control	0.731	0.725	0.745	0.705	0.708	0.772	0.747	0.745	0.774	0.749	0.726	0.786	0.742 def
0.00% L	0.716	0.713	0.730	0.688	0.713	0.767	0.743	0.743	0.778	0.745	0.730	0.783	0.738 f
0.50% L	0.729	0.729	0.756	0.718	0.731	0.782	0.752	0.746	0.786	0.750	0.719	0.782	0.748 bcde
1.00% L	0.717	0.713	0.726	0.698	0.708	0.767	0.740	0.735	0.781	0.752	0.717	0.783	0.736 f
2.00% L	0.738	0.733	0.755	0.709	0.723	0.785	0.760	0.767	0.795	0.740	0.733	0.798	0.755 ab
4.00% L	0.725	0.729	0.732	0.702	0.717	0.770	0.752	0.739	0.776	0.762	0.715	0.769	0.739 f
6.00% L	0.734	0.736	0.755	0.696	0.699	0.768	0.729	0.722	0.770	0.748	0.702	0.765	0.734 f
8.00% L	0.736	0.738	0.756	0.703	0.712	0.785	0.739	0.745	0.776	0.721	0.703	0.776	0.742 def
0.00% H	0.755	0.754	0.767	0.720	0.737	0.803	0.770	0.774	0.795	0.742	0.730	0.778	0.762 a
0.50% H	0.746	0.751	0.754	0.704	0.700	0.788	0.735	0.731	0.781	0.732	0.701	0.770	0.741 def
1.00% H	0.736	0.740	0.754	0.700	0.701	0.776	0.736	0.730	0.780	0.738	0.715	0.769	0.740 ef
2.00% H	0.743	0.749	0.753	0.712	0.722	0.788	0.749	0.760	0.788	0.741	0.726	0.779	0.752 bc
4.00% H	0.744	0.745	0.760	0.715	0.718	0.791	0.751	0.739	0.786	0.746	0.715	0.780	0.749 bcd
6.00% H	0.737	0.744	0.752	0.698	0.703	0.786	0.735	0.731	0.775	0.744	0.701	0.765	0.738 f
8.00% H	0.737	0.739	0.751	0.709	0.716	0.785	0.747	0.744	0.777	0.740	0.709	0.765	0.743 cdef
LSD	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.0094

[1]Treatment – Treatment column is representing the level of sugar in the solution indicated by the percentage and the rate of surfactant indicated by H or L. Where H = 1.00% and L = 0.25%

[2]NDVI – Normalized Difference Vegetative Index which assesses the amount of live green material in the sampling area where 0 = no green material and 1 = ideal turf.

Table A.5 Main effects of fructose and surfactants treatments on visual ratings for glasshouse study of 2010 for Cargill 42 High Fructose Corn Syrup.

	Turf Quality[1]													
	--Week--													
	Pre	1	2	3	4	5	6	7	8	9	10	11	12	Total
Treatment	--- 1 – 9 Scale ---													
Control	6.67	6.67	7.33	7.33	7.67	8.00	7.67	7.33	7.00	6.33	6.33	6.00	5.67	6.923 bcd
0.50% L	6.00	6.00	6.67	7.33	7.67	8.00	8.00	8.00	8.00	6.33	7.00	7.33	7.33	7.205 ab
1.00% L	6.67	7.00	7.33	7.67	8.00	8.00	8.00	7.67	8.00	6.67	6.67	6.67	6.67	7.308 ab
2.00% L	7.33	7.67	7.67	8.00	7.67	7.33	7.00	7.00	7.33	6.33	6.33	7.00	6.67	7.180 abc
4.00% L	6.33	7.00	6.67	7.33	7.67	8.00	8.00	8.00	8.00	7.33	8.00	8.00	7.33	7.513 a
6.00% L	6.33	7.00	7.00	7.33	7.33	8.00	7.67	7.33	7.67	7.00	7.33	7.67	6.67	7.256 ab
8.00% L	7.00	7.67	7.33	7.33	7.33	8.00	8.00	7.67	8.00	7.00	7.00	8.00	7.33	7.513 a
0.50% H	7.00	7.33	7.33	8.00	7.00	5.67	5.67	5.33	6.67	4.00	4.33	4.33	4.33	5.923 g
1.00% H	6.33	7.00	7.00	7.67	6.67	6.67	6.67	6.67	7.33	6.00	5.33	5.67	5.67	6.513 def
2.00% H	7.00	7.33	7.67	7.67	7.33	6.33	6.33	6.67	7.00	6.00	5.67	6.00	6.00	6.692 cde
4.00% H	6.33	7.00	6.67	7.33	7.33	5.67	6.33	6.00	6.33	5.67	5.00	5.00	5.33	6.154 fg
6.00% H	7.00	7.33	7.00	7.33	7.33	5.00	5.67	5.67	6.33	5.67	5.00	5.33	6.33	6.231 efg
8.00% H	6.67	7.67	7.00	7.00	7.00	5.33	5.67	6.00	6.00	6.33	6.33	6.00	6.33	6.410 efg
LSD														0.510

[1]Turf Quality was rated visually on a 1 to 9 scale, 1 = brown, dead turf, 7 was the minimum value for acceptable golf course putting green turf quality, 9 = ideal turf.

Table A.6 Main effects of fructose and surfactants treatments on NDVI readings for glasshouse study of 2010 for Cargill 42 High Fructose Corn Syrup.

	NDVI[2]													
	Week													
	Pre	1	2	3	4	5	6	7	8	9	10	11	12	Average
Treatment[1]	0 – 1 Scale													
Control	0.613	0.630	0.666	0.651	0.629	0.654	0.634	0.656	0.594	0.488	0.579	0.576	0.570	0.611 a
0.50% L	0.581	0.602	0.627	0.642	0.638	0.606	0.605	0.654	0.557	0.509	0.551	0.606	0.649	0.602 ab
1.00% L	0.656	0.660	0.678	0.677	0.655	0.617	0.573	0.627	0.579	0.510	0.574	0.598	0.628	0.618 a
2.00% L	0.676	0.700	0.693	0.693	0.699	0.579	0.542	0.567	0.561	0.483	0.573	0.637	0.638	0.618 a
4.00% L	0.603	0.662	0.669	0.654	0.655	0.593	0.576	0.584	0.564	0.496	0.584	0.608	0.627	0.606 a
6.00% L	0.614	0.656	0.669	0.638	0.634	0.572	0.550	0.538	0.523	0.460	0.578	0.605	0.601	0.587 abc
8.00% L	0.678	0.725	0.695	0.656	0.629	0.565	0.521	0.508	0.527	0.452	0.557	0.563	0.571	0.588 abc
0.50% H	0.667	0.699	0.733	0.711	0.601	0.482	0.450	0.438	0.497	0.452	0.427	0.504	0.500	0.551 cde
1.00% H	0.612	0.675	0.678	0.674	0.592	0.481	0.473	0.535	0.546	0.478	0.505	0.528	0.539	0.563 bcd
2.00% H	0.653	0.665	0.680	0.660	0.599	0.499	0.480	0.544	0.516	0.452	0.528	0.460	0.590	0.564 bcd
4.00% H	0.621	0.632	0.663	0.656	0.552	0.451	0.431	0.489	0.488	0.398	0.465	0.549	0.489	0.529 de
6.00% H	0.638	0.667	0.673	0.644	0.577	0.430	0.398	0.462	0.408	0.370	0.479	0.458	0.545	0.519 e
8.00% H	0.672	0.683	0.674	0.623	0.559	0.454	0.419	0.455	0.407	0.362	0.449	0.482	0.496	0.518 e
LSD														0.040

[1]Treatment – Treatment column is representing the level of sugar in the solution indicated by the percentage and the rate of surfactant indicated by H or L. Where H = 1.00% and L = 0.25%

[2]NDVI – Normalized Difference Vegetative Index which assesses the amount of live green material in the sampling area where 0 = no green material and 1 = ideal turf.

Table A.7 Main effects of fructose and surfactants treatments on visual ratings for glasshouse study of 2010 for Swanson 100% Pure Fructose.

	Turf Quality[2]													
	Week													
	Pre	1	2	3	4	5	6	7	8	9	10	11	12	Total
	1 – 9 Scale													
Treatment[1]														
Control	7.00	7.33	7.67	7.33	7.00	7.00	7.33	7.67	7.33	6.67	6.67	7.00	7.33	7.167 ab
0.50% L	7.33	7.33	7.33	8.00	7.67	7.33	6.67	6.33	6.67	7.00	6.67	7.00	7.67	7.191 a
1.00% L	6.67	6.67	7.00	7.00	7.33	7.33	6.33	6.33	6.67	7.00	6.67	6.67	7.33	6.857 bcd
2.00% L	6.33	6.33	7.00	7.33	7.67	7.67	6.67	6.67	6.67	6.67	6.33	6.67	7.33	6.881 abcd
4.00% L	7.00	7.00	7.00	7.00	7.67	7.00	7.00	6.67	7.00	7.33	6.67	7.00	7.33	7.071 abc
6.00% L	7.00	7.00	7.00	7.33	7.33	7.00	7.00	7.00	7.00	7.00	6.67	7.33	7.00	7.071 abc
8.00% L	7.00	6.67	6.67	7.00	7.33	7.00	7.00	6.33	7.00	6.67	6.67	7.00	7.33	6.929 abcd
0.50% H	5.67	5.33	5.67	6.00	6.00	6.00	5.33	5.00	5.00	5.33	5.67	5.67	6.67	5.714 e
1.00% H	7.33	7.33	7.33	7.33	7.33	6.67	6.67	7.00	6.33	6.67	6.67	7.33	7.33	7.048 abc
2.00% H	6.33	6.67	7.00	7.00	7.00	6.67	6.67	6.33	6.67	6.67	6.67	7.00	7.33	6.786 cd
4.00% H	6.67	7.00	6.67	6.67	7.67	7.00	7.00	7.00	7.00	7.00	6.67	7.33	7.67	7.071 abc
6.00% H	6.00	5.33	5.67	6.00	7.00	6.67	7.33	7.67	7.33	7.67	7.00	7.67	7.67	6.905 abcd
8.00% H	6.00	6.00	6.00	6.33	6.67	6.33	7.00	7.00	7.00	6.67	6.67	7.33	7.33	6.667 d
LSD														0.314

[1]Treatment – Treatment column is representing the level of sugar in the solution indicated by the percentage and the rate of surfactant indicated by H or L. Where H = 1.00% and L = 0.25%

[2]Turf Quality was rated visually on a 1 to 9 scale, 1 = brown, dead turf, 7 was the minimum value for acceptable golf course putting green turf quality, 9 = ideal turf.

Table A.8 Main effects of fructose and surfactants treatments on NDVI for glasshouse study of 2010 for Swanson 100% Pure Fructose.

	NDVI[2]													
	---Week---													
	Pre	1	2	3	4	5	6	7	8	9	10	11	12	Average
Treatment[1]	--- 0 – 1 Scale ---													
Control	0.684	0.708	0.721	0.712	0.716	0.638	0.648	0.676	0.637	0.625	0.534	0.623	0.648	0.659 a
0.50% L	0.695	0.684	0.710	0.742	0.731	0.666	0.613	0.627	0.599	0.601	0.552	0.616	0.671	0.654 ab
1.00% L	0.617	0.680	0.710	0.730	0.736	0.643	0.570	0.570	0.580	0.579	0.520	0.614	0.643	0.630 abc
2.00% L	0.632	0.697	0.695	0.743	0.721	0.695	0.615	0.634	0.633	0.597	0.558	0.615	0.631	0.651 ab
4.00% L	0.663	0.657	0.703	0.740	0.718	0.692	0.578	0.620	0.615	0.623	0.543	0.623	0.645	0.648 ab
6.00% L	0.670	0.695	0.692	0.721	0.717	0.681	0.616	0.655	0.606	0.577	0.523	0.605	0.632	0.646 ab
8.00% L	0.684	0.684	0.708	0.738	0.716	0.616	0.558	0.571	0.567	0.550	0.485	0.609	0.639	0.625 bc
0.50% H	0.589	0.598	0.595	0.654	0.673	0.529	0.510	0.504	0.482	0.503	0.504	0.572	0.607	0.563 d
1.00% H	0.684	0.590	0.707	0.742	0.733	0.625	0.562	0.609	0.577	0.592	0.552	0.632	0.662	0.636 abc
2.00% H	0.649	0.662	0.690	0.743	0.700	0.620	0.530	0.565	0.524	0.601	0.544	0.630	0.641	0.623 bc
4.00% H	0.692	0.603	0.697	0.742	0.722	0.644	0.571	0.643	0.582	0.598	0.554	0.630	0.656	0.641 abc
6.00% H	0.584	0.655	0.641	0.699	0.716	0.619	0.622	0.659	0.624	0.629	0.580	0.649	0.656	0.641 abc
8.00% H	0.630	0.641	0.670	0.673	0.681	0.569	0.572	0.602	0.560	0.564	0.530	0.606	0.622	0.609 c
LSD														0.032

[1]Treatment – Treatment column is representing the level of sugar in the solution indicated by the percentage and the rate of surfactant indicated by H or L. Where H = 1.00% and L = 0.25%

[2]NDVI – Normalized Difference Vegetative Index which assesses the amount of live green material in the sampling area where 0 = no green material and 1 = ideal turf.

Table A.9 The effect of fructose sources and surfactant on visual ratings for 2010 across both sources in regards to treatment.

Treatment[2]	Cargill	Swanson
	-------------------Turf Quality (1-9)[1]-------------------------	
Control	6.94 bcd	7.19 a
0.50% L	7.31 ab	7.14 a
1.00% L	7.36 ab	6.85 bcde
2.00% L	7.17 abc	6.92 abcd
4.00% L	7.61 a	7.06 abc
6.00% L	7.33 ab	7.06 abc
8.00% L	7.56 a	6.89 abcd
0.50% H	5.83 f	6.56 e
1.00% H	6.53 de	7.00 abcd
2.00% H	6.67 cde	6.81 cde
4.00% H	6.14 ef	7.06 abc
6.00% H	6.17 ef	6.92 abcd
8.00% H	6.39 e	6.92 abcd
Mean[3]	6.93	6.85

[1]Turf Quality was rated visually on a 1 to 9 scale, 1 = brown, dead turf, 7 was the minimum value for acceptable golf course putting green turf quality, 9 = ideal turf.
[2]Treatment – Treatment represents the level of sugar in the solution indicated by the percentage and the rate of surfactant indicated by H or L. Where H = 1.00% and L = 0.25%
[3]Mean corresponds to average turf quality across all weeks within a source. Different letters within the same column indicate significance at the 0.05 level (P<0.05).

Table A.10 The effect of fructose sources and surfactant on visual ratings for 2010 in regards to week.

WAT[2]	Cargill	Swanson
	--------------------Turf Quality (1-9)[1]--------------------	
1	7.13 abc	6.69 e
2	7.13 abc	6.85 cde
3	7.49 a	7.03 c
4	7.39 ab	7.28 ab
5	6.92 c	6.97 cd
6	6.97 bc	6.85 cde
7	6.87 c	6.77 de
8	7.21 abc	6.82 cde
9	6.21 d	6.87 cde
10	6.18 d	6.67 e
11	6.39 d	7.05 bc
12	6.28 d	7.36 a
Mean[3]	6.93	6.85

[1]Turf Quality was rated visually on a 1 to 9 scale, 1 = brown, dead turf, 7 was the minimum value for acceptable golf course putting green turf quality, 9 = ideal turf.
[2]Week after initial treatment.
[3]Mean corresponds to average turf quality across all weeks within a source. Different letters within the same column indicate significance at the 0.05 level ($P<0.05$).

Table A.11 The effect of fructose sources and surfactant on NDVI measurement for 2010 across both sources in regards to treatment.

Treatment[2]	Cargill	Swanson	Mean[3]
	-------------------------- NDVI[1] (0-1) ----------------------		
Control	0.611 a	0.657 a*	0.634 a
0.50% L	0.604 abc	0.651 ab*	0.627 ab
1.00% L	0.615 a	0.631 cd	0.623 ab
2.00% L	0.614 a	0.653 ab*	0.633 a
4.00% L	0.606 ab	0.646 abc*	0.626 ab
6.00% L	0.585 bc	0.644 abc*	0.614 bc
8.00% L	0.581 cd	0.620 de*	0.600 cd
0.50% H	0.541 ef	0.606 e*	0.574 f
1.00% H	0.559 de	0.632cd*	0.595 de
2.00% H	0.556 e	0.621 de*	0.588 def
4.00% H	0.522 fg	0.637 bcd*	0.579 ef
6.00% H	0.509 g	0.646 abc*	0.577 f
8.00% H	0.505 g	0.608 e*	0.556 g
Mean[4]	0.575	0.633 *	

[1]NDVI – Normalized Difference Vegetative Index which assesses the amount of live green material in the sampling area where 0 = no green material and 1 = ideal turf.

[2]Treatment – Treatment column represents the level of sugar in the solution indicated by the percentage and the rate of surfactant indicated by H or L. Where H = 1.00% and L = 0.25%

[3]Mean corresponds to average NDVI across sources within a treatment. Different letters within a column indicate significance at the 0.05 level (P<0.05).

[4]Mean corresponds to average NDVI across treatments within a source. An asterisk within a row indicates significantly higher NDVI at specified treatment level at the 0.05 level of significance (P<0.05).

Table A.12 The effect on fructose sources and surfactant on NDVI measurement for 2010 across both treatment in regards to week.

WAT[2]	Cargill	Swanson	Mean[3]
	------------------------- NDVI (0-1)[1]	--------------------------	
1	0.666 a	0.656 c	0.661 b
2	0.677 a	0.695 b	0.686 a
3	0.660 a	0.729 a*	0.694 a
4	0.617 c	0.720 a*	0.669 b
5	0.537 de	0.639 d*	0.588 d
6	0.512 f	0.585 f*	0.549 e
7	0.543 de	0.615 e*	0.579 d
8	0.521 ef	0.584 g*	0.552 e
9	0.455 g	0.590 f*	0.522 f
10	0.527 ef	0.538 g	0.532 f
11	0.552 cd	0.620 e*	0.586 d
12	0.573 c	0.645 cd*	0.609 c
Mean[4]	0.570	0.635*	

[1]NDVI – Normalized Difference Vegetative Index which assesses the amount of live green material in the sampling area where 0 = no green material and 1 = ideal turf.
[2]Week after initial treatment.
[3]Mean corresponds to average NDVI across sources within a week. Different letters within a column indicate significance at the 0.05 level (P<0.05).
[4]Mean corresponds to average NDVI across dates within a source. An asterisk within a row indicate significantly higher NDVI at specified treatment level at the 0.05 level of significance (P<0.05).