

**Influence of mowing frequency and mower sharpness on efficiency of PSII and  
antioxidant and carbohydrate metabolism of creeping bentgrass**

by

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### ABSTRACT

Sports fields are mown primarily to provide a uniform surface for ball roll and bounce. However, mowing creates openings that facilitate water loss and entry points for pathogens, and increases susceptibility to other stresses. The objectives of our research were to identify physiological mechanisms that allow creeping bentgrass to tolerate close and frequent mowing. Plants often increase formation of reactive oxygen species (ROS) in response to wounding. Accumulation of ROS may damage macromolecules such as lipids, proteins, and nucleic acids. Damage to these macromolecules may result in reduced plant growth and vigor, or even death. Plants form enzymes to quench ROS and protect cells from oxidative damage. However, activities of ROS-scavenging enzymes often are reduced during stress. In our research, activities of the ROS-quenching enzymes catalase and ascorbate peroxidase were reduced in mowed grasses. Reduced activities of these enzymes may result in

accumulation of ROS. However, no differences were observed in levels of lipid peroxidation between not-cut and mowed grasses, indicating that accumulation of ROS was not sufficient to cause severe oxidative stress. Although toxic at high concentrations, ROS may act as a component of a signal transduction pathway that acclimates not-stressed tissue to potential stress. It is necessary for grasses to regrow leaf tissue removed by mowing. Reserve carbohydrates often are hydrolyzed to generate carbon for respiration and to provide substrates for development of leaf and shoot tissue. Creeping bentgrass forms fructans as reserve carbohydrates. In our research, fructans were reduced in mown grasses compared to not-cut grasses. Glucose levels also were reduced in mowed grasses compared to not-cut grasses. Glucose likely was oxidized in the glycolytic pathway and respiration to provide energy for formation of new leaf tissue. It is important to remember that mowing is a stress and that mowing programs should balance agronomic requirements of grasses with sports play demands in order to produce uniform and visually appealing turf that is vigorous in growth.



## **GENERAL INTRODUCTION**

### **Thesis Organization**

This thesis is organized into five chapters. The first chapter is a general introduction to topics relevant to this thesis and a review of pertinent literature. The second chapter is a manuscript titled ‘Activities of antioxidant enzymes, efficiency of PSII, and lipid peroxidation in mowed creeping bentgrass.’ The third chapter is a manuscript titled ‘Carbohydrate content and efficiency of PSII in mown creeping bentgrass.’ The fourth chapter is a manuscript titled ‘Quality of cut of creeping bentgrass as influenced by mower sharpening method.’ The fifth chapter is a review of the results and general conclusions drawn from the manuscripts.

### **Literature Review**

Edwin Budding patented the first mechanical lawn mower in 1830. Similar in design to the nap-cutting equipment used in the textile factory in which he worked, Budding’s mower used a rotating cylinder of blades and a stationary bedknife to cut grass (Bell, 1995). Six blades were welded in a helical pattern around a central shaft to form the cylinder, or reel. As the reel rotated, it gathered grass to a bedknife positioned below the reel that clipped grass as the mower was propelled forward (Bell, 1995). Modern mowers differ from Budding’s original design only in the material used to manufacture them. Early reel mowers were constructed by using wrought and cast iron, whereas newer mowers are made of steel (Bell, 1995).

Mowing is necessary to form dense and visually appealing playing surfaces that facilitate movement of athletes as well as ball roll and bounce (Christians, 2004). Grasses, however, do not flourish as a result of mowing; rather, they merely tolerate the process. Frequent and

close mowing predisposes grasses to pathogen attack and increases susceptibility to environmental stresses (León et al., 2001). Although reel-type mowers have been used for over 150 years, little research has addressed development of practical mowing programs that balance agronomic requirements of grasses with sports play demands.

Most of the research that has dealt with mowing has examined changes in morphology or visual quality of grasses in response to cutting height. Juska and Hanson (1961) observed that low mowing heights (2.5 cm) reduced leaf regrowth of Kentucky bluegrass (*Poa pratensis* L.) and root production. Leaves of Kentucky bluegrass often assume a more horizontal orientation and form more leaves per tiller in response to low mowing heights (2.5 cm) to maximize photosynthetic leaf area (Sheffer et al., 1978). Several researchers have observed that visual quality of creeping bentgrass (*Agrostis stolonifera* L.) is reduced as mowing heights are lowered (Fagerness et al., 2000; Razmjoo et al., 1996; Sailaiz et al., 1995). In general, root growth decreases as mowing height decreases (Juska and Hanson, 1961; Krans and Beard, 1985). Kentucky bluegrass (*Poa pratensis* L.) clipped weekly at 6.2 and 2.5 cm developed 41 and 76% less root mass, respectively, than non-mown controls two to four weeks after the first mowing (Krans and Beard, 1985). Liu and Huang (2002) observed shorter total root length of creeping bentgrass (*Agrostis stolonifera* L.) mown at 3 mm compared with grasses mown at 4 mm. Fagerness and Yelverton (2001) also noticed a reduction of root mass in creeping bentgrass mowed at 3.2 mm compared to turf clipped at 4.0 or 4.8 mm.

However, little research has examined physiological responses that allow grasses to tolerate close and frequent mowing. In this dissertation, we wanted to identify mechanisms that grasses use to perceive and acclimate to mowing stress, as well as indicators of plant health and recuperative potential after mowing.

Plants often form reactive oxygen species (ROS) in response to wounding. Accumulation of ROS may damage macromolecules such as lipids, proteins, and nucleic acids (Asada, 1999). Damage to these macromolecules may result in reductions in rates of photosynthesis and respiration. Because of the highly toxic nature of reactive oxygen species, plants have developed enzymatic systems to scavenge ROS and protect cells from oxidative damage (Mittler, 2002).

Ascorbate peroxidase (APX), catalase (CAT), glutathione reductase (GR), and superoxide dismutase (SOD) are important enzymes that detoxify ROS. The SOD enzyme catalyzes the conversion of  $O_2^-$  to  $H_2O_2$ , whereas APX and CAT catalyze the conversion of  $H_2O_2$  to water. Ascorbate peroxidase and glutathione reductase (GR) scavenge  $H_2O_2$  in the ascorbate-glutathione cycle (Foyer and Halliwell, 1976). Activities of ROS-scavenging enzymes often are reduced during stress conditions such as drought, high temperature, chilling, wounding, and pathogen attack, however, resulting in accumulation of ROS and oxidative stress (Dat et al., 2000).

Mowing limits  $CO_2$ -assimilation by removing leaf tissue that plants use to intercept quantum energy (White, 1973). Regrowth and development of new leaf tissue after mowing is necessary to form the photosynthetic leaf area required for normal plant growth and development (Parsons et al., 1983). Grasses often increase leaf and shoot density below the height-of-cut to reestablish photosynthetic leaf surface area (Sheffer et al., 1978). Plants use carbohydrate reserves to provide energy and raw materials required to redevelop leaf tissue and form tillers.

Fructans are the primary reserve carbohydrates formed in creeping bentgrass (*Agrostis stolonifera* L.). Fructans are water-soluble polymers of fructose stored in leaf sheaths and elongating leaf bases (Chatterton, 1989). Decreased levels of fructans have been observed in

grasses in response to defoliation, likely caused by hydrolysis of carbohydrate reserves for transport to actively growing tissue (Morvan-Bertrand et al., 2001; Prud'homme et al., 1992; Volenec, 1986). Labeling studies have demonstrated fructose hydrolyzed from fructans is transported as sucrose to growing leaves in defoliated perennial grasses (Amiard et al., 2003). The duration and magnitude of reduction in levels of reserve carbohydrates often is dependent on the severity of defoliation. In general, lower mowing heights and more frequent cuttings cause greater reductions in carbohydrate reserves (Donaghy and Fulkerson, 1998).

Regular mower maintenance is essential to achieve the best possible quality of cut. Dull mower blades tear and fray leaf blades, resulting in necrotic and ragged leaf tips that reduce visual quality of grasses. Moreover, grasses mown with dull mowers are subjected to more stress than grasses mown with sharp mowers (Howieson, 2001). Several methods of sharpening reel-type mowers exist, including single-blade grinding, spin-grinding, carbide-milling, back-lapping, and facing of the front face of the bedknife.

The decision about which method, or combination of methods, to use often depends on economic, labor, and time factors, as well as desired cutting quality and personal preference. There is no set standard regarding how often reel-type mowers should be sharpened. That depends on several factors, such as the area of turf to be mowed, the regularity of cutting, the species of grass being cut, the method used to sharpen the mower, and the adjustment of the reel to the bedknife.

Grasses maintained on sports fields often are double-cut, or clipped twice in the same mowing, to create a more uniform playing surface and improve ball roll (Nikolai, 2004). This practice is especially prevalent on golf course greens. However, it is not known if

multiple cuttings are more damaging to growth, development, and vigor of creeping bentgrass (*Agrostis stolonifera* L.) compared to single-cutting.

The primary objective of this research was to determine if double-cutting is more damaging to plant growth and development than single-cutting. A secondary objective was to determine how frequently mowers should be sharpened to achieve the highest quality of cut. Measurements of activities of reactive oxygen species-scavenging enzymes, carbohydrate concentrations, chlorophyll content, efficiency of photosynthesis, leaf blade tissue damage, and lipid peroxidation were used to quantify mowing stress and injury.

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**ACTIVITIES OF ANTIOXIDANT ENZYMES, EFFICIENCY OF PSII,  
AND LIPID PEROXIDATION IN MOWED CREEPING BENTGRASS**

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**Abstract**

The objective of this study was to determine if multiple cuttings damage creeping bentgrass (*Agrostis stolonifera* L) growth and development more than single-cutting or rolling. Formation of reactive oxygen species (ROS) is a common response of plants to wounding. Accumulation of ROS may damage macromolecules, reduce rates of photosynthesis and respiration, and eventually cause cell death. Because of the highly toxic nature of ROS, plants have developed enzymatic systems to scavenge ROS and protect cells from oxidative damage. Catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR), and superoxide dismutase (SOD) are important ROS-quenching enzymes. Activities of APX, CAT, GR, and SOD were assayed over time following rolling, single-cutting, and double-cutting. Efficiency of PSII and lipid peroxidation were measured to quantify oxidative injury. Activities of APX and CAT were transiently reduced in rolled, single-cut, and double-cut grasses, which may result in accumulation of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). However, accumulation of H<sub>2</sub>O<sub>2</sub> was not sufficient to cause lipid peroxidation, likely because of increased GR reductase activity. Decreases in efficiency of PSII were observed in rolled, single-cut, and double-cut grasses, suggesting reduced efficiency and inhibition of photosynthesis. Duration and level of changes observed in antioxidant enzyme activities and efficiency of PSII of double-cut grasses suggest that multiple cuttings are more injurious to plant growth than rolling or single-cutting.

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Abbreviations: APX, ascorbate peroxidase; CAT, catalase; GR, glutathione reductase; H<sub>2</sub>O<sub>2</sub>, hydrogen peroxide; MDA, malondialdehyde; ROS, reactive oxygen species; SOD, superoxide dismutase

Additional Index Words: ascorbate peroxidase, catalase, glutathione reductase, malondialdehyde, efficiency of PSII, superoxide dismutase

## Introduction

Grasses regularly are mowed to maintain uniform playing surfaces on sports fields. Mowing, however, is a stress that removes large amounts of leaf tissue and opens wounds that facilitate water loss and are potential sites for pathogen invasion. A common response of many plants to wounding is to increase formation of reactive oxygen species (ROS) (Orozco-Cárdenas and Ryan, 1999).

Reactive oxygen species are reduced derivatives of oxygen (O<sub>2</sub>) that damage carbohydrates, lipids, nucleic acids, and proteins (Asada, 1999). Common ROS formed by plants include superoxide radicals (O<sub>2</sub><sup>-</sup>), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), and hydroxyl radicals (HO<sup>-</sup>) (Dat et al., 2000). Accumulation of ROS may damage cellular components, inhibit rates of photosynthesis and respiration, and ultimately lead to cell death. Plants have developed protective mechanisms that detoxify ROS to limit their accumulation and avoid oxidative stress (Dat et al., 2000).

Plants form several antioxidant enzymes, including ascorbate peroxidase (APX), catalase (CAT), and superoxide dismutase (SOD), that quench ROS. The SOD enzyme catalyzes the conversion of O<sub>2</sub><sup>-</sup> to H<sub>2</sub>O<sub>2</sub>, whereas APX and CAT catalyze the conversion of H<sub>2</sub>O<sub>2</sub> to water. Ascorbate peroxidase works in conjunction with dehydroascorbate reductase (DHAR) and glutathione reductase (GR) to scavenge H<sub>2</sub>O<sub>2</sub> in the ascorbate-glutathione cycle (Foyer and Halliwell, 1976). Dehydroascorbate reductase and GR

maintain cellular pools of reduced forms of the antioxidant compounds ascorbate and glutathione (Nakano and Asada, 1981). Stress conditions often reduce activities or levels of antioxidant enzymes, exacerbating ROS accumulation (Dat et al., 2000).

Grasses often are double-cut, or cut twice in the same mowing, to improve the playability and visual appearance of sports fields. Rolling also is a common cultural practice used to improve the consistency and speed of sports fields by using a weighted drum or cylinder to smooth and compact the grass surface. It has not been established if double-cutting is more damaging to grass health than single-cutting or rolling. The objective of this study was to quantify mowing stress by using antioxidant enzyme activities, lipid peroxidation, and efficiency of PSII as indicators of plant stress. We hypothesized that double-cut grasses would have the largest decrease in activities of antioxidant enzymes and efficiency of PSII and greatest increase in lipid peroxidation compared to not-cut grasses.

### **Materials and Methods**

Seeds of 'L-93' creeping bentgrass (*Agrostis stolonifera* L.) were sown at a rate of 24 kg ha<sup>-1</sup> in 12.7 cm wide by 11.4 cm deep pots of sand in a greenhouse. Temperature was 18-25 °C and natural sunlight was supplemented with high-pressure sodium lamps that provided 200 μmol m<sup>-2</sup> s<sup>-1</sup> of photosynthetic active radiation (PAR) and a photoperiod of 16/8 (day/night). Sand was watered daily and fertilized weekly with 110 -mL of a complete nutrient solution that contained 102 mg L<sup>-1</sup> nitrogen, 4 mg L<sup>-1</sup> phosphorus, and 63 mg L<sup>-1</sup> potassium (Pellet and Roberts, 1963). Grasses were mowed three times a week at 1.3 cm. A wood box was built that held grasses for mowing. The box was ramped at both ends to facilitate the use of an electric greens mower (Toro, Inc., Minneapolis, MN)

to cut grasses. After a minimum of eight months grasses were transferred to a growth chamber at 21/18 °C (day/night), 550  $\mu\text{mol m}^{-2} \text{s}^{-1}$  of PAR, and a photoperiod of 16/8 (day/night). Grasses were maintained and mowed in the growth chamber for three weeks before application of treatments.

Grasses were allowed to grow uncut for three days prior to the initiation of mowing treatments. Grasses either were rolled with an 108 kg electric greens mower operated without the reel engaged (rolled), mowed once at 1.3 cm (single-cut), or mowed twice in the same direction at 1.3 cm (double-cut). Control grasses were not rolled or mowed. Measurements of antioxidant enzyme activity, lipid peroxidation, and efficiency of PSII were made 3, 6, 12, and 24 hours after treatment. Leaf tissue was collected at each sampling time, frozen under liquid nitrogen, and stored at -80 °C for analysis of antioxidant enzyme activity and lipid peroxidation.

Efficiency of photosystem II (PSII) of individual leaves was measured by using a chlorophyll fluorometer (PAM-101, Walz, Effeltrich, Germany) fitted with a 2 mm fiber optic. Estimates of efficiency of PSII were determined from  $F_m' - F' / F_m'$ , where  $F_m'$  is fluorescence in the light-adapted state and  $F'$  is initial fluorescence before measuring  $F_m'$  (Genty et al., 1989).

Frozen leaf tissue (0.5 g) was crushed under liquid  $\text{N}_2$  and homogenized with 10-mL of solution that contained 50 mM potassium phosphate buffer (pH 7.0), 1% insoluble polyvinylpyrrolidone, 1mM EDTA, and 0.2 mM ascorbic acid (ASC) to extract soluble proteins. Supernatant was collected for enzyme assays after the homogenate was centrifuged at 15000 g for 20 min at 4 °C. Protein concentration was determined according to Bradford (1976) by using bovine serum albumin as a standard.

Ascorbate peroxidase (EC 1.11.1.11) activity was assayed by following the oxidation of ascorbate over time. The 1-mL reaction mixture contained 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM ASC, 0.1 mM H<sub>2</sub>O<sub>2</sub>, and 200  $\mu$ L enzyme extract. The reaction was initiated by addition of supernatant and the decrease in absorbance at 290 nm was monitored for 1 min with a spectrophotometer (Beckman Coulter DU 640, Fullerton, CA). Activity of APX was calculated by using an extinction coefficient of 2.8 mM<sup>-1</sup> cm<sup>-1</sup> (Nakano and Asada, 1981).

Catalase (EC 1.11.1.6) activity was determined by using the method of Chance and Maehly (1955). Two hundred microliters of supernatant were added to a 3-mL reaction mixture that contained 50 mM potassium phosphate buffer (pH 7.0), and 15 mM H<sub>2</sub>O<sub>2</sub>. Consumption of H<sub>2</sub>O<sub>2</sub> was followed at 240 nm for 1 min. A decrease of 0.01 units of absorbance per min was defined as one unit of CAT activity (Liu and Huang, 2000).

Glutathione reductase (EC 1.6.4.2) activity was determined by following the oxidation of NADPH at 340 nm for 1 min. The 1-mL reaction mixture contained 50 mM potassium phosphate buffer (pH 7.8), 2 mM Na<sub>2</sub>EDTA, 0.15 mM NADPH, 0.5 mM GSSG, and 100  $\mu$ L of supernatant. The activity of GR was calculated by using an extinction coefficient of 6.2 mM<sup>-1</sup> cm<sup>-1</sup> (Schaedle and Bassham, 1977).

Activity of SOD (EC 1.15.1.1) was assayed by using the method of Giannopolitis and Ries (1977). Reaction mixtures contained 50 mM potassium phosphate buffer (pH 7.8), 13 mM methionine, 75  $\mu$ M nitro blue tetrazolium (NBT), 0.1 mM EDTA, 2  $\mu$ M riboflavin, and 20-60  $\mu$ L supernatant. Reaction mixtures were placed under 15 watt fluorescent lamps with a light intensity of 70  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup> PAR for 10 min. Reaction mixtures that were not illuminated developed no color and served as blanks, whereas illuminated reaction mixtures that lacked supernatant developed maximum color and

served as positive controls. Reduction of NBT was measured at 560 nm. One unit of SOD was defined as the amount of enzyme needed to inhibit reduction of NBT by 50%

Malondialdehyde (MDA) content was used to estimate lipid peroxidation (Dhindsa et al., 1981). One gram of frozen leaf tissue was homogenized with 2-mL of ice-cold 0.1% (w/v) trichloroacetic acid (TCA) and centrifuged for 10 min at 15000 g. A 1-mL aliquot of extract was mixed with 2-mL of a 20% (w/v) TCA and 0.5% (w/v) thiobarbituric acid solution and heated at 95 °C for 30 min. The mixture was cooled on ice and centrifuged at 10000 g for 10 min. Malondialdehyde content was calculated by using absorbencies of supernatant at 532 and 600 nm and an extinction coefficient of 155 mM<sup>-1</sup> cm<sup>-1</sup> (Heath and Packer, 1968; Liu and Huang, 2000).

Treatments were completely randomized. Activities of antioxidant enzymes and MDA content were determined at each sampling time from three independent replicates per treatment. Measurements of efficiency of PSII were made from ten individual leaf blades of each sampled pot prior to tissue harvest. The experiment was replicated twice in time. The general linear model of SAS (SAS Institute, Cary, NC) was used for analysis of variance of experiment, treatment, time, and their interaction effects. Because of a significant experiment x treatment x time interaction ( $P \leq 0.05$ ), data are reported separately for each experiment. Treatment means were separated by using pairwise comparisons.

## Results

Efficiency of PSII of rolled grasses were reduced by as much as 6 and 5%, respectively, compared to not-cut grasses in experiments one and two (Fig. 1). In both experiments, the efficiency of PSII of rolled grasses was similar to not-cut grasses 6 hours after treatment. Efficiency of PSII of single-cut grasses were reduced by 4%

compared to not-cut grasses 12 hours after treatment in experiment one (Fig. 1a), whereas Efficiency of PSII were reduced by 6% 6 hours after treatment in experiment two (Fig. 1b). In experiment one, efficiency of PSII of double-cut grasses was reduced by 11% compared to not-cut grasses 12 hours after treatment (Fig. 1a), whereas in experiment two efficiency of PSII was reduced by 5% 12 hours after treatment(Fig. 1b).

Greatest reduction in CAT activity in experiment one was observed in double-cut grasses 12 hours after treatment. Compared to not-cut grasses, activity of CAT was reduced by 39% in double-cut grasses (Fig. 2). Catalase activity of rolled grasses was lower than not-cut grasses 6 hours after treatment in experiment one, whereas activities were similar to not-cut grasses in experiment two (Fig. 2)

Decreases in ascorbate peroxidase (APX) activities were observed in rolled, single-cut, and double-cut grasses 3 hours after treatment in experiment one (Fig. 3). Compared to not-cut grasses, activity of APX was reduced by 24 % in rolled grasses, whereas the activities of APX were reduced by 27% in single- and double-cut grasses. Activities of APX were similar among all treatments for the remainder of the experiment. In experiment two, APX activities were reduced 6 hours after treatment in single- and double-cut grasses and then increased above the level of not-cut grasses 12 hours after treatment (Fig. 3). Activities of APX respectively increased by 7 and 21 % in single- and double-cut grasses compared to not-cut grasses.

Glutathione reductase (GR) activity increased by 49 and 34%, respectively, in single- and double-cut grasses 3 hours after treatment in experiment one (Fig. 4). Twelve hours after treatment, GR activities of rolled and double-cut grasses had increased above the level of not-cut grasses. Activity of GR in double-cut grasses increased compared to not-cut grasses 12 hours after treatment in experiment two (Fig 4). Glutathione reductase

activity of double-cut grasses increased by 37 and 41% compared to not-cut grasses in experiment one and two, respectively, 12 hours after treatment.

No differences in superoxide dismutase (SOD) activity were observed in either experiment one or two (data not shown). Likewise, levels of lipid peroxidation, measured in terms of malondialdehyde content, were unchanged among treatments in both experiments (data not shown).

### **Discussion**

Because no differences were observed in lipid peroxidation among not-cut, rolled, single-cut, and double-cut grasses, it is unlikely that ROS accumulated to levels necessary to cause severe oxidative damage. However, decreases in efficiency of PSII were observed in rolled, single-cut, and double-cut grasses, suggesting photoinhibition of photosystem II. Reduction of efficiency of PSII also has been observed in wounded *Arabidopsis* (Chang et al., 2004). Photoinhibition results when light energy absorbed exceeds the capacity of photosynthetic electron transport. Although an indicator of stress, photoinhibition often is reversible and considered a mechanism that prevents overexcitation of the photosynthetic system and concomitant non-reversible oxidative damage, lipid peroxidation, and cell death (Long et al., 1994).

During normal conditions, ROS formed during metabolic processes such as photosynthesis and respiration are scavenged by antioxidant systems (Møller, 2001; Krieger-Liszkay, 2004). However, when plants are subjected to stresses such as drought, high temperature, chilling, wounding, and pathogen attack, scavenging systems often are overwhelmed, resulting in accumulation of ROS (Mittler, 2002). In this study, activities of CAT and APX were reduced in rolled, single-cut, and double-cut grasses. Reduced

activities of these enzymes could result in accumulation of  $H_2O_2$  in wounded grasses. Although toxic to cells at high concentrations,  $H_2O_2$  has been ascribed a signaling role that acclimates not-stressed tissue to potential stress (Neil et al., 2002). Karpinski et al. (1999) observed that treatment with  $H_2O_2$  reduced oxidative stress in *Arabidopsis* leaves exposed to excess light energy. It was suggested that  $H_2O_2$  is a signaling component that induces stress-protective mechanisms in remote tissues to limit damage (Karpinski et al., 1999).

Mowing not only damages plant tissues, but opened wounds are susceptible to pathogens (Cheong et al., 2002). Accumulation of  $H_2O_2$  has been implicated in pathogen and insect responses. It has been observed that ROS form physical barriers to pathogens by strengthening cell walls adjacent to wounded tissue (Delledonne et al., 1998). Reactive oxygen species also are messengers that activate expression of defense-genes such as proteinase inhibitors (Orozco-Cárdenas et al., 2001).

Although  $H_2O_2$  may be used to perpetuate stress signals, the level of  $H_2O_2$  must be regulated to avoid over-accumulation and cell death. Activities of GR increased in single- and double-cut grasses after mowing. Increased expression of GR genes following stress previously have been reported in *Arabidopsis* and pea (Hernández et al., 2004; Karpinski, 1997). In this study, elevated GR activity coincided with recovered or increased activities of APX, although APX activity initially decreased in mowed grasses. In addition to quenching  $H_2O_2$  in the ascorbate-glutathione cycle, APX and GR maintain the antioxidants ascorbate and glutathione in their active, reduced state (Noctor and Foyer, 1998). Increased activities of APX and CAT, as well as increased levels of reduced glutathione and ascorbate, likely quenched  $H_2O_2$  to prevent damaging oxidative stress and lipid peroxidation (Asada and Takahashi, 1987).

Data from this experiment suggest that mowing alters antioxidant metabolism. The duration and magnitude of changes observed in antioxidant enzyme activities and efficiency of PSII of double-cut grasses indicate that multiple cuttings are more injurious to plant growth than rolling or single-cutting. Although double-cutting may improve the uniformity of sports fields, caution should be used, especially during adverse environmental conditions, because grasses that have been double-cut may be less vigorous in growth and more susceptible to other stresses.

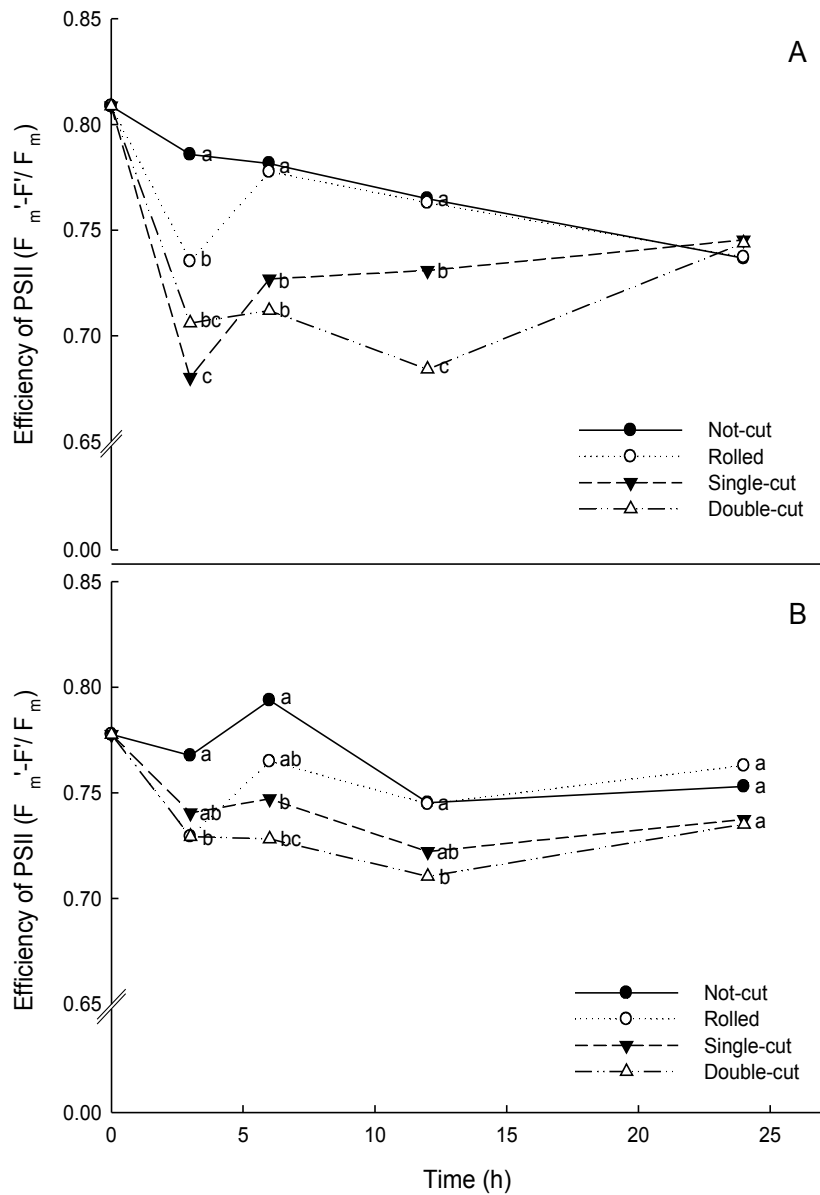
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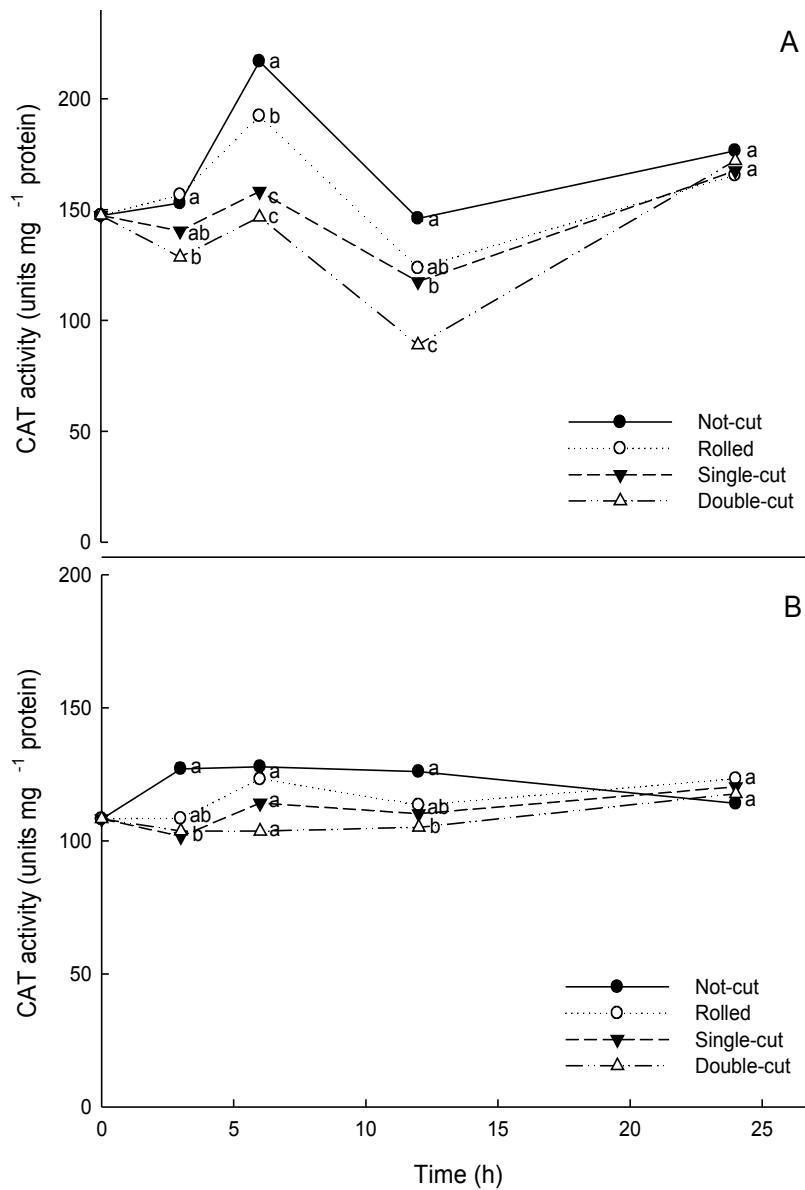
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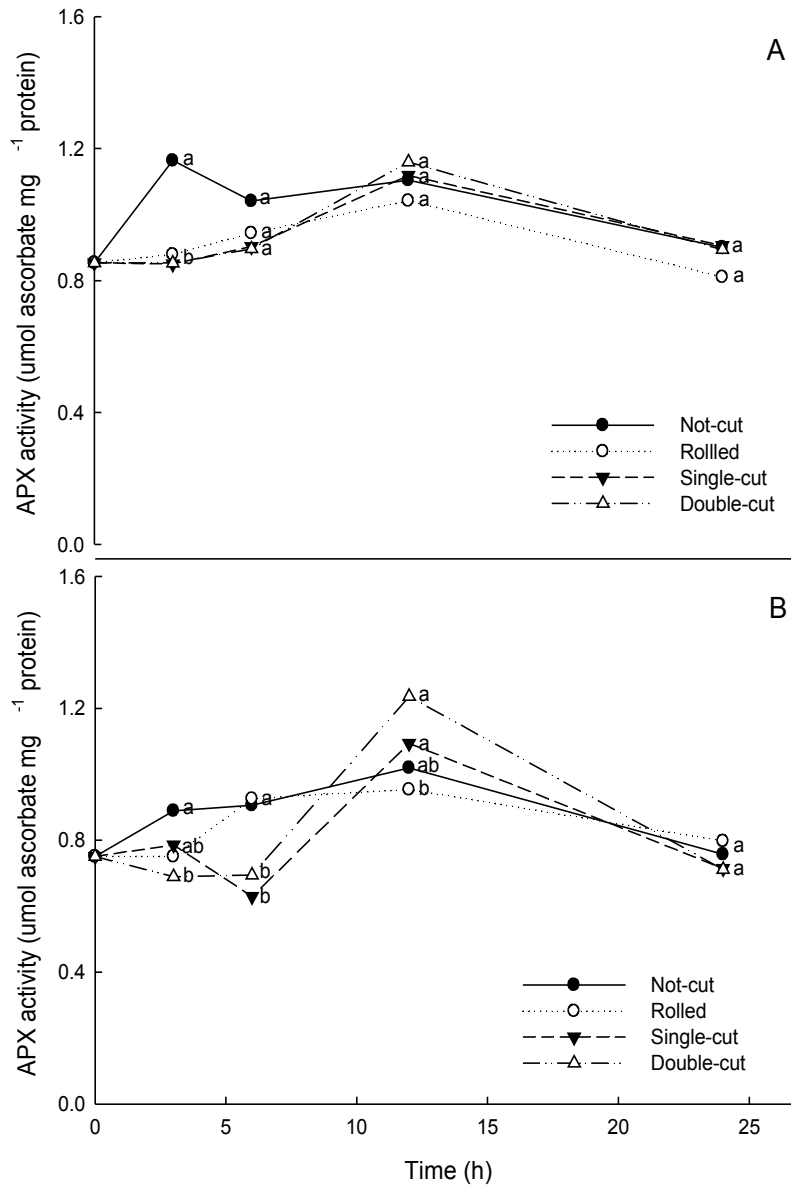
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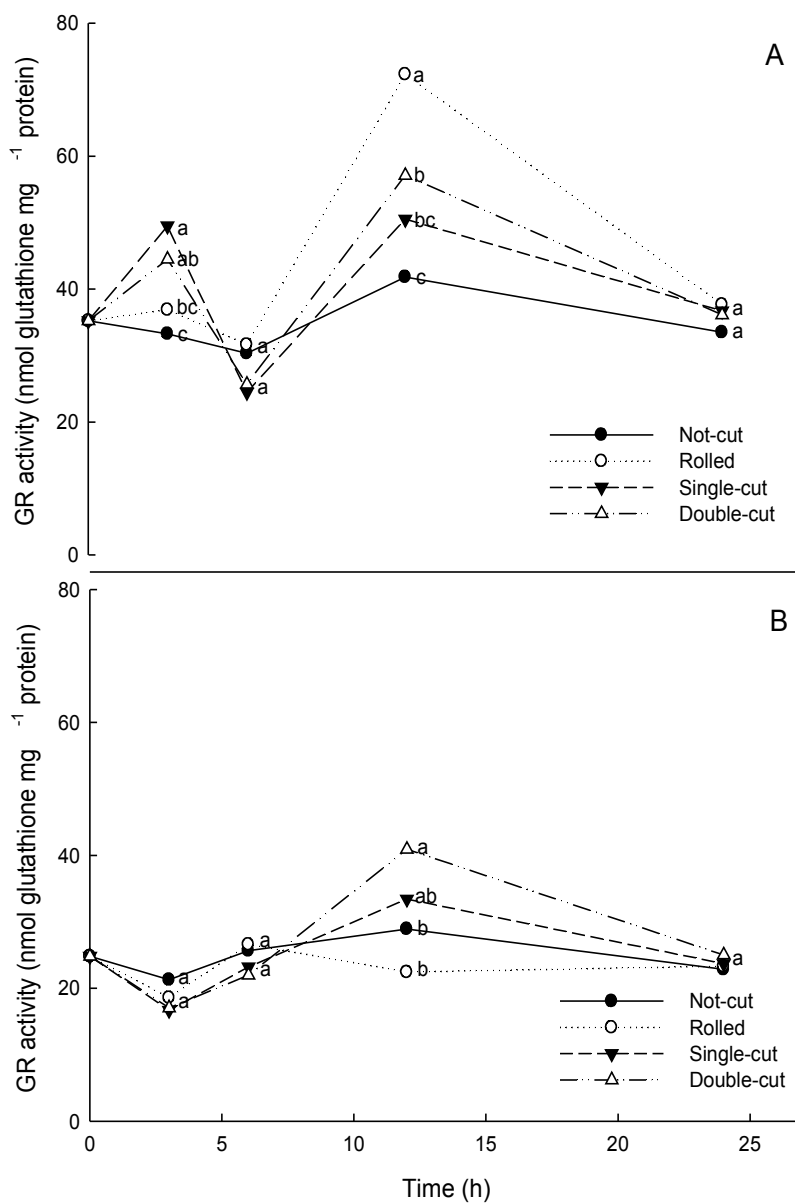
**Fig. 1.** Efficiency of PSII of not-cut, rolled, single-cut, and double-cut 'L-93' creeping bentgrass over time in experiment one (A) and experiment two (B). Means at each sampling time labeled with the same letter are not different at the 0.05 level by using pairwise comparisons to separate means. Each data point is the mean of 30 observations.



**Fig. 2.** CAT activity of not-cut, rolled, single-cut, and double-cut ‘L-93’ creeping bentgrass over time in experiment one (A) and experiment two (B). A decrease of 0.01 units of absorbance at 240 nm per min was defined as one unit of CAT activity. Means at each sampling time labeled with the same letter are not different at the 0.05 level by using pairwise comparisons to separate means. Each data point is the mean of 3 observations.



**Fig. 3.** APX activity of not-cut, rolled, single-cut, and double-cut ‘L-93’ creeping bentgrass over time in experiment one (A) and experiment two (B). Means at each sampling time labeled with the same letter are not different at the 0.05 level by using pairwise comparisons to separate means. Each data point is the mean of 3 observations.



**Fig. 4.** GR activity of not-cut, rolled, single-cut, and double-cut 'L-93' creeping bentgrass over time in experiment one (A) and experiment two (B). Means at each sampling time labeled with the same letter are not different at the 0.05 level by using pairwise comparisons to separate means. Each data point is the mean of 3 observations.

## CARBOHYDRATE METABOLISM AND EFFICIENCY OF PSII IN MOWN CREEPING BENTGRASS

M.J Howieson and N.E. Christians\*

### Abstract

Regrowth of leaf tissue after mowing is necessary to form photosynthetic leaf area required for CO<sub>2</sub>-assimilation and plant growth and development. Leaf tissue regrowth often is dependent on levels of reserve carbohydrates stored in leaf sheath and leaf bases. The objective of this study was to quantify mowing injury by measuring levels of fructan, sucrose, glucose, fructose, and efficiency of PSII in not-cut, rolled, single-cut, and double-cut creeping bentgrass grown in a greenhouse. Efficiency of PSII was reduced in double-cut grasses by as much as 9% compared to not-cut grasses. Fructan levels were reduced in single- and double-cut grasses compared to not-cut grasses by 52 and 45%, respectively, 36 hours after mowing. Glucose levels were 31% lower in double-cut grasses compared to not-cut grasses. No differences were observed in sucrose and fructose levels among not-cut, rolled, single-cut, and double-cut grasses. Mowing transiently reduced fructan and glucose levels in mowed grasses. Duration of reduction of fructan levels and magnitude of reduction in glucose levels and efficiency of PSII were greatest in double-cut grasses, suggesting that multiple cuttings may be more damaging to plant vigor than single-cutting.

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Abbreviations: EDTA, ethylenediamine tetraacetic acid; HPLC, high performance liquid chromatography

Additional Index Words: *Agrostis stolonifera* L., fructose, glucose, sucrose, double-cut, rolled, single-cut

## Introduction

Mowing removes leaf tissue that grasses use to acquire solar energy, thereby limiting photosynthesis and carbon-assimilation (White, 1973). Regrowth and initiation of new leaf tissue after mowing is necessary to develop photosynthetic leaf area required for production of carbohydrates (Parsons et al., 1983). Rate of leaf tissue regrowth often is dependent on levels of carbohydrates present in grass tissue prior to defoliation, suggesting that plants rely on carbohydrate reserves to provide energy and raw materials to redevelop leaf and shoot tissue (Donaghy and Fulkerson, 1998; Morvan-Betrand et al., 1997; Davidson and Milthorpe, 1966).

The primary reserve carbohydrate of creeping bentgrass (*Agrostis stolonifera* L.) is fructan. Fructan is a polymer of fructose with a terminal glucose moiety (Chatterton, 1989). Increased catabolism and decreases in levels of fructans have been observed in grasses in response to defoliation (Morvan-Betrand et al., 2001; Prud'homme et al., 1992; Volenec, 1986). The magnitude of reduction in levels of reserve carbohydrates often is dependent on the severity of defoliation. In general, lower mowing heights and more frequent cuttings cause greater reductions in carbohydrate reserves (Donaghy and Fulkerson, 1998). Fructose hydrolyzed from fructan can be converted to the monosaccharide glucose by isomerase enzymes, or used to form the disaccharide sucrose, which is the primary sugar transported in phloem. Mono- and disaccharides formed from products of fructan hydrolysis are used for respiration and development of new foliage following defoliation (Amiard et al., 2003; Morvan-Betrand et al., 1999).

Playing surfaces often are double-cut or rolled to improve uniformity and increase ball roll (Nikolai, 2004). Double-cutting is clipping grasses twice in the same mowing, whereas

grasses are rolled by using lightweight drums or cylinders to lightly compact the grass surface (Nikolai, 2004). It is not known if multiple cuttings limit the recuperative potential of mowed grasses more than single-cutting or rolling. The objective of this study was to quantify mowing injury by measuring levels of fructan, sucrose, glucose, fructose, and efficiency of PSII in not-cut, rolled, single-cut, and double-cut grasses.

### Materials and Methods

Creeping bentgrass (*Agrostis stolonifera* L.) cv. L-93 seeds were planted at a rate of 24 kg ha<sup>-1</sup> in 12.7 cm wide by 11.4 cm deep pots of sand and grown in a greenhouse. Greenhouse temperatures were 18-25 °C with a photoperiod of 16/8 (day/night). Natural sunlight was supplemented with high pressure sodium lamps that provided an additional 180 μmol m<sup>-2</sup> s<sup>-1</sup> of photosynthetic active radiation (PAR). Sand was watered daily. Grasses were cut at 1.3 cm three times a week with an electric greens mower (Toro, Inc., Minneapolis, MN). A wood box was built with ramps on either side that allowed the electric greens mower to cut grasses. Sand media was fertilized once a week with 110-mL of a complete nutrient solution described by Pellet and Roberts (1963) that contained 102 mg L<sup>-1</sup> nitrogen, 4 mg L<sup>-1</sup> phosphorus, and 63 mg L<sup>-1</sup> potassium. Grasses were moved to a growth chamber ten months after seeding. Grasses were mowed, watered, and fertilized as in the greenhouse. Temperature in the growth chamber was 18-21 °C and a photoperiod of 16/8 (day/night) was maintained with cool white fluorescent lamps and incandescent bulbs that provided 550 μmol m<sup>-2</sup> s<sup>-1</sup> of PAR. Grasses were acclimated in the growth chamber for three weeks prior to initiation of treatments.

Grasses were not cut for three days prior to application of mowing treatments. Mowing treatments included rolling, single-cutting, and double-cutting. Grasses were rolled with a

108 kg electric greens mower without the reel engaged, whereas single-cut grasses were mowed once and double-cut grasses were mowed twice at 1.3 cm. Harvests of plant tissue for carbohydrate analysis and measurements of efficiency of PSII were made 12, 24, 36, 48, and 60 hours after treatment. A portable fluorometer (PAM-101, Walz, Effeltrich, Germany) was used to measure light-adapted efficiency of PSII ( $(F_m' - F') / F_m'$ ) of individual leaf blades, where  $F_m'$  is fluorescence in the light-adapted state and  $F'$  is initial fluorescence (Genty et al., 1989). Leaf tissue collected at each sampling time was immediately frozen under liquid nitrogen and stored at -80 °C for analysis of carbohydrates.

Frozen leaf samples were thawed at 4 °C and a subsample was dried in a 68 °C oven for 72 hours for dry weight analysis. A 0.5 to 1.0 g sample of leaf tissue was crushed under liquid N<sub>2</sub> and suspended in 10-mL of HPLC-grade water to extract soluble carbohydrates. The mixture was heated to 95 °C for 30 minutes and then centrifuged at 1000 g for 10 minutes (Livingston, 1990). Samples were deionized by passing a 100 µL aliquot of supernatant through a 1-mL chromatography column packed from top to bottom with 120 µL cation exchange resin (Dowex 50 x8-400, H<sup>+</sup>-form), 100 µL insoluble polyvinylpyrrolidone, and 120 µL anion exchange resin (Amberlite CG-400 II, formate-form). Columns were eluted twice with 250 µL water. Elutants were combined and filtered through a 0.45 µm nylon filter for HPLC analysis (Amiard et al., 2003; Bachmann, 1994).

Water soluble carbohydrates were analyzed by using high performance liquid chromatography (HPLC). Sucrose, glucose, fructose, and fructans were separated on a Sugar-Pak column (300 x 6.5 mm, Waters, Milford, MA) and were isocratically eluted at 85 °C by using 0.1 mM disodium calcium salt of EDTA at a flow rate of 0.5-mL min<sup>-1</sup>. Separated carbohydrates were identified by external standards and quantified by using a model 2410 differential refractometer (Waters, Milford, MA).

The experimental design was completely randomized. Three independent replicates were used to determine concentrations of carbohydrates at each sampling time. Efficiency of PSII was measured from ten individual leaves selected at random from pots prior to tissue sampling. The experiment was replicated twice in time. The general linear model of SAS (SAS Institute, Cary, NC) was used for analysis of variance of experiment, treatment, time, and their interactions. Values reported in the text and figures are representative of the highest order interaction or main effects significant at the 0.05 level. Treatment means were separated by using pairwise comparisons.

## Results

Efficiency of PSII of single-cut and rolled grasses were reduced by 5% compared to not-cut grasses 12 hours after treatment in experiment one (Fig. 1a). Double-cut grasses had a reduction in efficiency of PSII of 9% compared to not-cut grasses 12 hours after treatment in experiment one (Fig. 1a). Twenty-four hours after treatment, efficiency of PSII of single- and double-cut grasses were reduced by 4% in experiment one (Fig. 1a). In experiment two, single- and double-cut grasses had reductions in efficiency of PSII of 2 and 2.5%, respectively, compared to not-cut grasses 12 hours after treatment (Fig. 1b). Efficiency of PSII of single- and double-cut grasses were reduced by 3% compared to not-cut grasses 36 hours after treatment in experiment two (Fig. 1b).

Levels of fructan were averaged over experiment. Fructan levels were 33% lower in double-cut grasses compared to not-cut grasses 24 hours after treatment. Single-cut grasses had 22% less fructan than not-cut grasses 24 hours after treatment (Fig. 2). Thirty-six hours after treatment, fructan levels of single- and double-cut grasses were 52 and 45% lower than not-cut grasses. Fructan levels were 38% lower in double-cut grasses compared to not-cut

grasses 48 hour after treatment (Fig. 2). Glucose levels were averaged over experiment and time. Level of glucose was 31% lower in double-cut grasses compared to not-cut grasses (Fig. 3).

No differences were observed in levels of sucrose or fructose among treatments in either study (data not shown).

### **Discussion**

Efficiency of PSII consistently was lowest in double-cut grasses, although reductions in efficiency of PSII also were observed in rolled and single-cut grasses. Efficiency of PSII is used as an indicator of plant stress. Decreases in efficiency of PSII often are interpreted that applied stress has damaged the photosynthetic apparatus, and reduced efficiency of absorbed light transfer in photosynthetic electron transport (Maxwell and Johnson, 2000).

Levels of fructan were reduced in single- and double-cut grasses compared to rolled and not-cut grasses. Reduced levels of fructans also have been observed in mown perennial ryegrass (*Lolium perenne* L.) (Donaghy and Fulkerson, 1998; Sullivan and Sprague, 1943) and tall fescue (*Festuca arundinacea* Scrb.) (Volenec, 1986). Amiard et al. (2001) and de Visser (1997) have demonstrated that products of fructan hydrolysis are used for respiration and development of new leaf tissue until foliage has regrown and rate of CO<sub>2</sub>-assimilation is sufficient to support plant growth and metabolism. Magnitude of reductions in the levels of reserve carbohydrates often corresponds to severity of stress, suggesting that levels of carbohydrates can be used as an indicator of stress severity and recuperative potential (Morvan-Bertrand et al., 2001; Donaghy and Fulkerson, 1998).

The largest reductions of fructan levels were observed 36 hours after mowing in single- and double-cut grasses. However, duration of reduction of fructan levels was longer in

double-cut grasses. Levels of fructan in single-cut grasses were similar to not-cut grasses 48 hours after mowing, whereas fructan levels of double-cut grasses were similar to not-cut grasses 60 hours after mowing. Mowed grasses, regardless of mowing frequency, were able to regrow sufficient leaf tissue to support plant metabolism and restore levels of reserve carbohydrates. Narra et al. (2004) also observed similar carbohydrate levels in creeping bentgrass cut at different heights in clippings collected 4 to 6 days after mowing. However, adverse environmental conditions like high temperatures that also reduce levels of reserve carbohydrates may limit recuperative potential of mown creeping bentgrass (Xu and Huang, 2000).

Glucose levels also were reduced in single- and double-cut grasses compared to rolled and not-cut grasses. Levels of glucose were lowest in double-cut grasses compared to not-cut grasses. Glucose likely was oxidized in the glycolytic pathway to provide energy for formation of new leaf tissue.

No differences were observed in levels of sucrose or fructose. It is likely that fructose levels were maintained in single- and double-cut grasses by fructan hydrolysis (Prud'homme et al., 1992). Sink tissue demand likely maintained sucrose levels. Sucrose is the primary sugar translocated in the phloem (Taiz and Zeiger, 2002). Products of fructan hydrolysis in source tissues likely were used to form sucrose for transport to elongating leaf bases where sucrose was hydrolyzed into glucose and fructose by invertase to be used for respiration and formation of new leaf tissue.

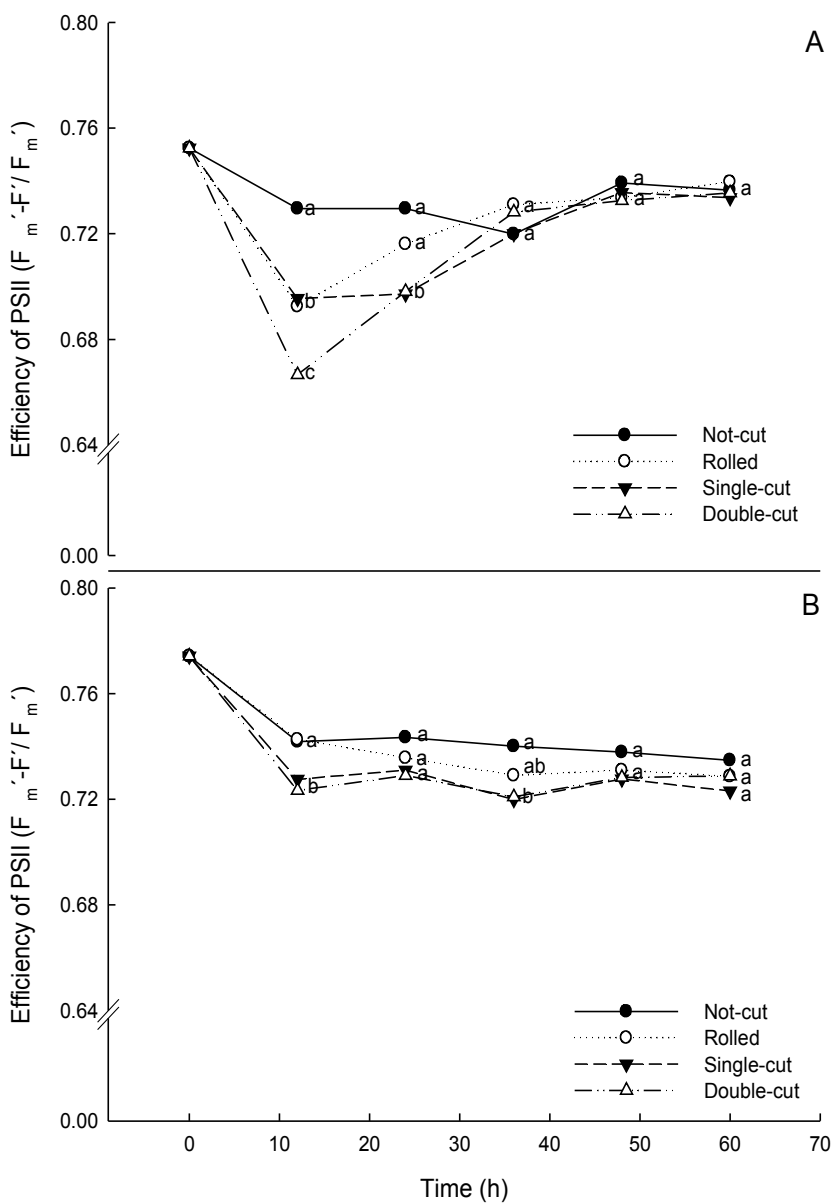
Mowing reduces efficiency of PSII and results in transient decreases in the levels of fructan and glucose in creeping bentgrass. The duration of reduction in fructan levels was longest in double-cut grasses, indicating that multiple cuttings may be more damaging to plant growth and development

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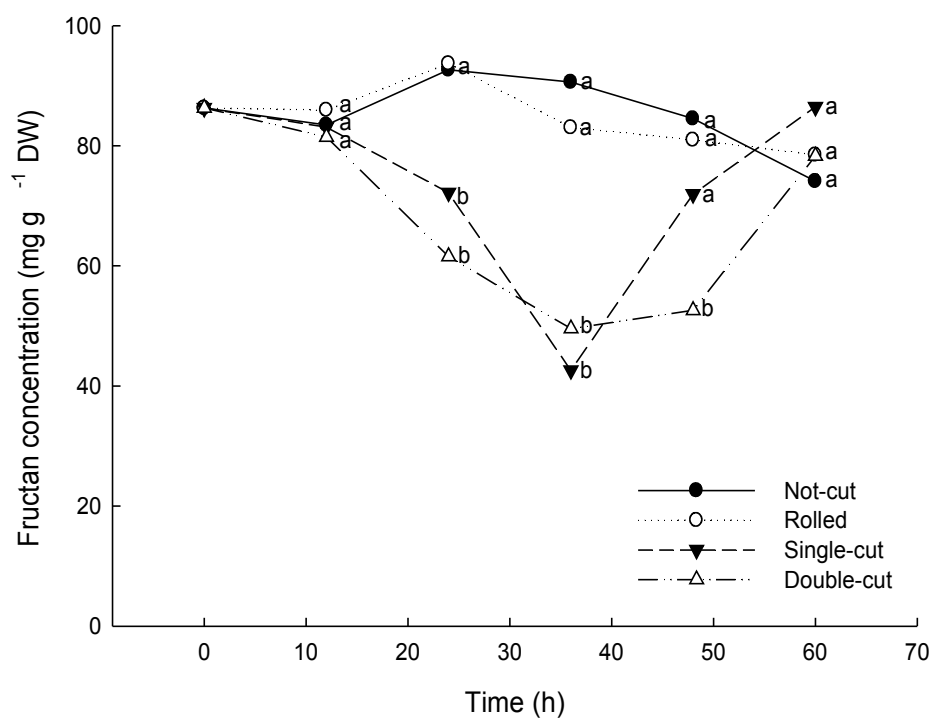
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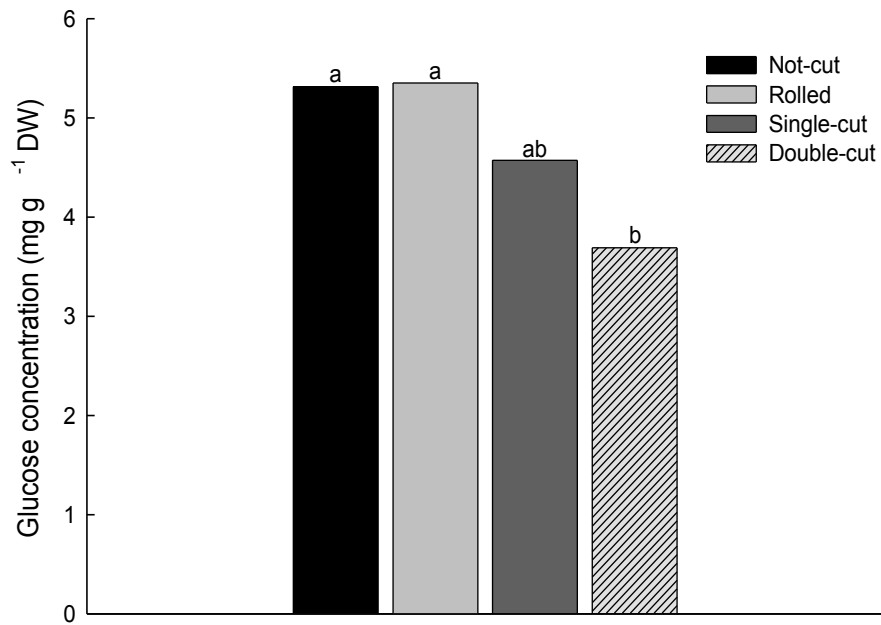
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**Fig. 1.** Efficiency of PSII of not-cut, rolled, single-cut, and double-cut 'L-93' creeping bentgrass over time in experiment one (A) and experiment two (B). Means at each sampling time labeled with the same letter are not different at the 0.05 level by using pairwise comparisons to separate means. Each data point is the mean of 30 observations.



**Fig. 2.** Fructan concentration of not-cut, rolled, single-cut, and double-cut ‘L-93’ creeping bentgrass. Data averaged over experiments. Means at each sampling time labeled with the same letter are not different at the 0.05 level by using pairwise comparisons to separate means. Each data point is the mean of 6 observations.



**Fig. 3.** Glucose concentration of not-cut, rolled, single-cut, and double-cut ‘L-93’ creeping bentgrass. Data averaged over experiment and time. Means labeled with the same letter are not different at the 0.05 level by using pairwise comparisons to separate means. Each data point is the mean of 30 observations.

## QUALITY OF CUT OF CREEPING BENTGRASS AS INFLUENCED BY MOWER SHARPENING METHOD

M.J Howieson and N.E. Christians\*

### Abstract

The objective of this study was to determine how frequently reel-type mowers should be sharpened to maintain high quality of cut. Mowers were sharpened by using single-blade carbide milling, cylindrical grind, and cylindrical with back grind methods. Mowers were used to cut 0.2-0.4 ha of 'L-93' creeping bentgrass (*Agrostis stolonifera* L.) every week for 12 weeks at Cold Water Golf Links (Ames, IA). Height of cut was 1.3 cm. Measurements of leaf blade tissue damage, estimated rate of photochemistry, and chlorophyll content were used to quantify mower sharpness over time. Leaf blade tissue damage was as much as 20-fold greater in grasses cut with cylindrical grind sharpened mowers compared to grasses cut with frequently-sharpened control mowers. Estimated rate of photochemistry was as much as 15% greater in grasses cut with sharpened control mowers compared to grasses cut with mowers sharpened by using the cylindrical grind method. These results suggest that mowers sharpened by using the cylindrical grind method need to be sharpened more frequently than mowers sharpened by using cylindrical with back grind and carbide milling methods to achieve high quality of cut.

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### Introduction

Sports fields are mowed to accommodate play of activities such as baseball, football, golf, soccer, and tennis. Mowing creates a uniform surface for movement of athletes as well as ball roll and bounce. Lawns and sports fields also are mowed for aesthetic reasons. Grasses that are properly mowed form a dense, uniform, and visually appealing turf (Christians, 2004). Sports fields often are cut twice in the same mowing, or double-cut, to improve uniformity of the playing surface. However, frequent wounding alters plant growth and development, predisposes wounded plants to pathogen attack, and increases susceptibility to environmental stresses (León et al., 2001). It is important, therefore, that appropriate mowing practices are developed to ensure plant health and vigor.

Regular mower maintenance is essential to achieve the best possible quality of cut. Dull mowers tear and shred leaf tissue rather than cutting it, severely wounding the plant (Howieson and Christians, 2001). Moreover, shredded or torn leaf tips become necrotic degrading visual appearance of mowed grasses. It is important to maintain mower blade and bedknife sharpness to produce the best possible quality of cut, limiting the occurrence of ragged and necrotic leaf tips (Benson, 1963; Howieson and Christians, 2001).

There is no set standard regarding how often reel-type mowers should be sharpened. That depends on several factors, such as the area of grass to be mowed, regularity of cutting, species of grass being cut, method used to sharpen the mower, and adjustment of the reel to the bedknife. The objectives of this study were to quantify mower sharpness and mowing injury over time of single- and double-cut creeping bentgrass (*Agrostis stolonifera* L.). This information will be used to determine how frequently reel-type mowers should be sharpened to achieve the best possible quality of cut of creeping bentgrass.

## **Materials and Methods**

Three walk-behind greens mowers (GR 800, Toro, Inc., Minneapolis, MN) were sharpened once by using either single-blade carbide milling (Trion Lifts, Inc., Evans, CO) cylindrical grind (Bernhard and Company Ltd., Rugby, England), or cylindrical with back grind (Foley United, River Falls, WI) methods by respective company representatives. Each mower was used to cut approximately 0.2-0.4 ha of 'L-93' creeping bentgrass (*Agrostis stolonifera* L.) every week for twelve weeks at Cold Water Golf Links (Ames, IA). Creeping bentgrass was mowed at a height of 1.3 cm. Mowers sharpened by using the cylindrical with back grind method after every 0.35 ha were used as positive controls. Regularly-sharpened positive control mowers were used to make comparisons to the other mowers as they dulled over time. Mowers sharpened by using cylindrical with back grind and carbide milling methods were adjusted prior to each mowing with slight reel-to-bedknife contact, whereas mowers sharpened by using cylindrical grind method were adjusted with no reel-to-bedknife contact. The gap between the reel and bedknife of these mowers was adjusted by using paper supplied by the manufacturer of the sharpening equipment.

Measurements of leaf blade tissue damage, estimated rate of photochemistry, and chlorophyll concentration were used to quantify quality of cut and mowing injury. Leaf blade tissue damage was the length of necrotic and torn leaf tissue was measured under a microscope (20x) 24 hours after mowing (Howieson and Christians, 2001). Rate of photochemistry was estimated from  $F_m' - F / F_m' \times$  photosynthetic photon flux density (PPFD) (Gentry, 1989). Chlorophyll fluorescence parameters  $F_m'$  and  $F$  were measured by using a portable chlorophyll fluorometer (PAM 101, Walz, Effeltrich, Germany) 6 to 9 hours after mowing. Chlorophyll was extracted from 1 g fresh weight leaf tissue in 10-mL of 80% acetone in the dark. Acetone extractions were repeated three times, supernatants pooled, and

chlorophyll concentrations of pooled supernatants were determined according to Bruinsma (1961).

Treatments were arranged in a split-plot with sharpening method as the whole-plot and frequency of cut, i.e. single- or double-cut, as the sub-plot. Treatments were replicated three times. Repeated measurements of leaf blade tissue damage, estimated rate of photochemistry, and chlorophyll content were made on each sharpening method by frequency of cut replicate. Data were analyzed by using the MIXED procedure of Statistical Analysis Software (SAS Institute, Cary, NC). Compound symmetry covariance structure best described leaf blade tissue damage, estimated rate of photochemistry, and chlorophyll content data based on criteria proposed by Littell et al. (1998). Means were separated by using pairwise comparisons.

## **Results**

Grasses single-cut with mowers sharpened by using the cylindrical grind method formed as much as 4-fold greater leaf blade tissue damage than grasses cut by sharpened control mowers (Fig. 1). No differences in leaf tissue damage were observed between grasses single-cut with mowers sharpened by using the carbide milling method and sharpened control mowers until over 3.5 ha of grass had been mowed (Fig. 1). Leaf blade tissue damage was 2-fold greater in grasses clipped with mowers sharpened by using the carbide milling method compared to sharpened controls at this time. Leaf blade tissue damage of single-cut grasses sharpened by using the cylindrical with back grind method was nearly 3-fold greater than leaf blade tissue damage of sharpened control mowers after 3.0 ha of grass was mowed (Fig. 1). Double-cut grasses clipped with mowers sharpened by using the cylindrical grind method

formed necrotic tissue 3 to 7.5-fold greater than grasses cut with sharpened control mowers (Fig. 2).

Compared to grasses double-cut with sharpened control mowers, grasses double-cut with mowers sharpened by using the cylindrical grind method had 8% lower estimated rates of photochemistry (Fig. 3). The highest estimated rates of photochemistry of single- and double-cut grasses were observed in grasses cut with sharpened control mowers (Fig. 3). However, estimated rates of photochemistry were 2% lower in double-cut grasses mown with sharpened control mowers compared to single-cut grasses. Estimated rates of photochemistry were reduced by 2% in double-cut grasses clipped with mowers sharpened by using the carbide milling methods compared to single-cut grasses (Fig. 3). Double-cut grasses mowed with mowers sharpened by using the cylindrical grind method had 2% lower estimated rates of photochemistry compared to single-cut grasses (Fig.3).

Chlorophyll contents of grasses cut with mowers sharpened by using the carbide milling method and sharpened control mowers were similar (Fig 4.) Grasses cut with mowers sharpened by using cylindrical grind method contained 13% less chlorophyll than grasses mowed with sharpened control mowers (Fig. 4). Double-cut grasses contained 8% less chlorophyll than single-cut grasses (Fig. 4).

### **Discussion**

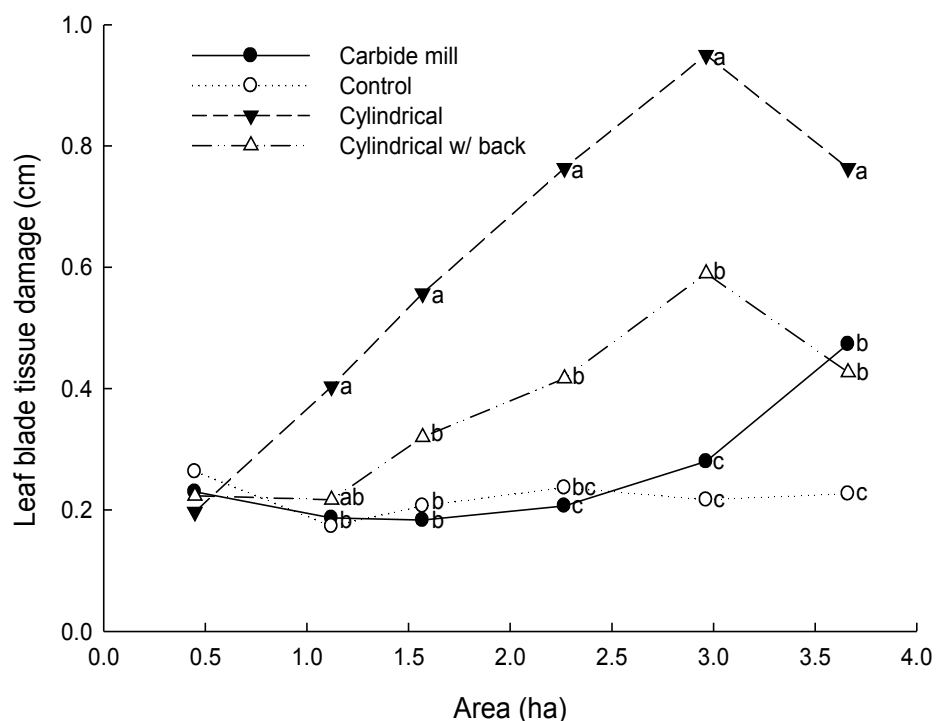
Grasses cut with mowers sharpened by using the cylindrical grind method consistently had the largest leaf blade tissue damage. Mowers sharpened by using the cylindrical grind method were adjusted with no reel-to-bedknife contact as recommended by the sharpening equipment manufacturer. When the reel and bedknife dulled, leaf blades likely were pulled across the bedknife and torn, rather than being cut with a scissors-like action. Reduced

chlorophyll content and estimated rate of photochemistry observed in grasses cut with mowers sharpened by using the cylindrical grind method suggests that this sharpening method is more damaging to plant growth and development than cylindrical with back grind and carbide milling methods. These results indicate that mowers sharpened by using the cylindrical grind method would need to be sharpened frequently, perhaps after every hectare of grass mown, to maintain acceptable quality of cut. Based on leaf blade tissue damage, mowers sharpened by using cylindrical with back grind method would need to be re-sharpened after every 1.5 to 2.5 ha of grass mowed, whereas mowers sharpened by using the carbide milling method would need to be sharpened after every 3.5 ha of grass mowed.

Double-cut grasses generally had less leaf blade tissue damage compared to single-cut grasses, whereas estimated rates of photochemistry and chlorophyll content were reduced in double-cut grasses. Reel-type mowers cannot position leaf blades before cutting. If a leaf blade is not positioned perpendicular to the bedknife before being cut, the likelihood that the leaf blade will be torn or only partially cut increases (Benson, 1963). Mowing grasses twice in the same mowing, or double-cutting, increases the probability that leaf blades will be positioned correctly in relation to the bedknife and removes partially-cut leaf blade tips from the first-cutting. These results suggest that double-cutting may improve quality of cut, but increases severity of mowing stress compared to single-cutting.

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**Fig. 1.** Leaf blade tissue damage (cm) over time of single-cut 'L-93' creeping

bentgrass. Length of necrotic and frayed tissue was measured under a microscope (20x).

Control mowers were sharpened after every 0.35-ha of grass was mowed by using the

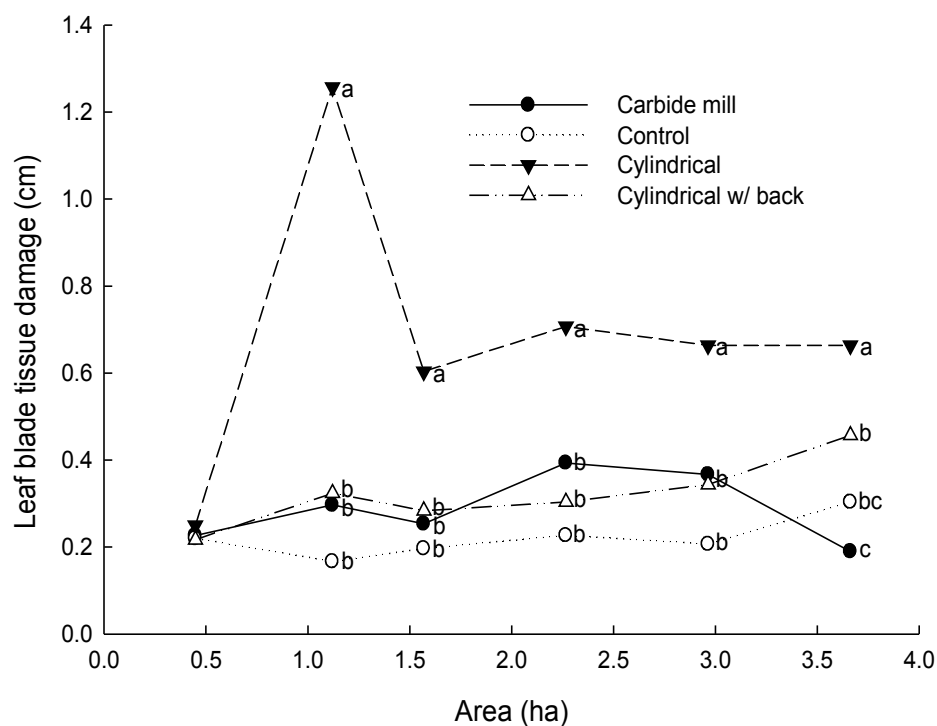
cylindrical with back grind method. Creeping bentgrass was clipped with mowers sharpened

by using cylindrical grind, cylindrical with back grind, and carbide milling methods. Each

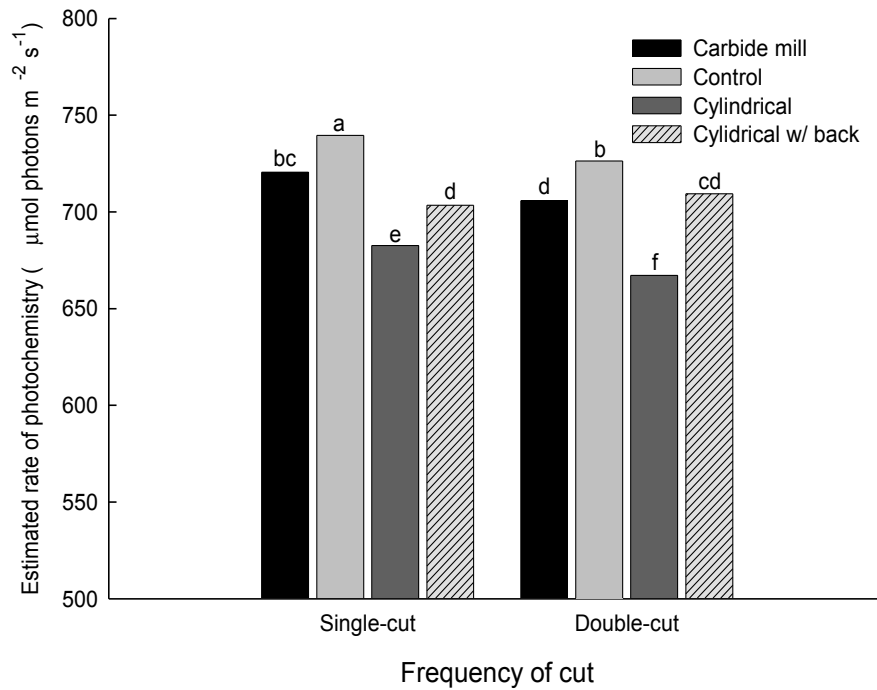
data point is the mean of 30 observations. Means labeled with the same letter at each

sampling time are not different at  $P \leq 0.05$ . Means were separated by using pairwise

comparisons.

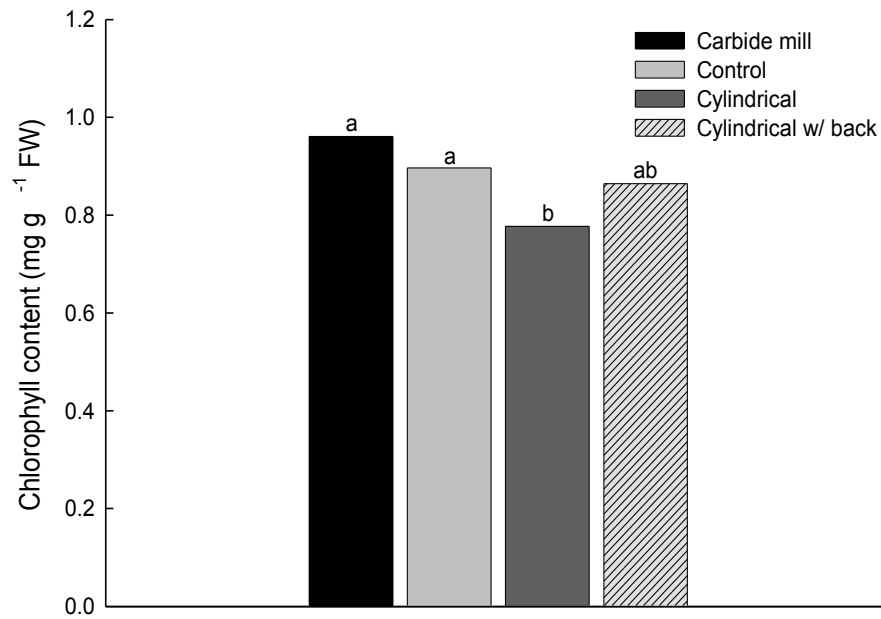


**Fig. 2.** Leaf blade tissue damage (cm) over time of double-cut ‘L-93’ creeping bentgrass. Length of necrotic and frayed tissue was measured under a microscope (20x). Control mowers were sharpened after every 0.35-ha of grass was mowed by using the cylindrical with back grind method. Creeping bentgrass was clipped with mowers sharpened by using cylindrical grind, cylindrical with back grind, and carbide milling methods. Each data point is the mean of 30 observations. Means labeled with the same letter at each sampling time are not different at  $P \leq 0.05$ . Means were separated by using pairwise comparisons.



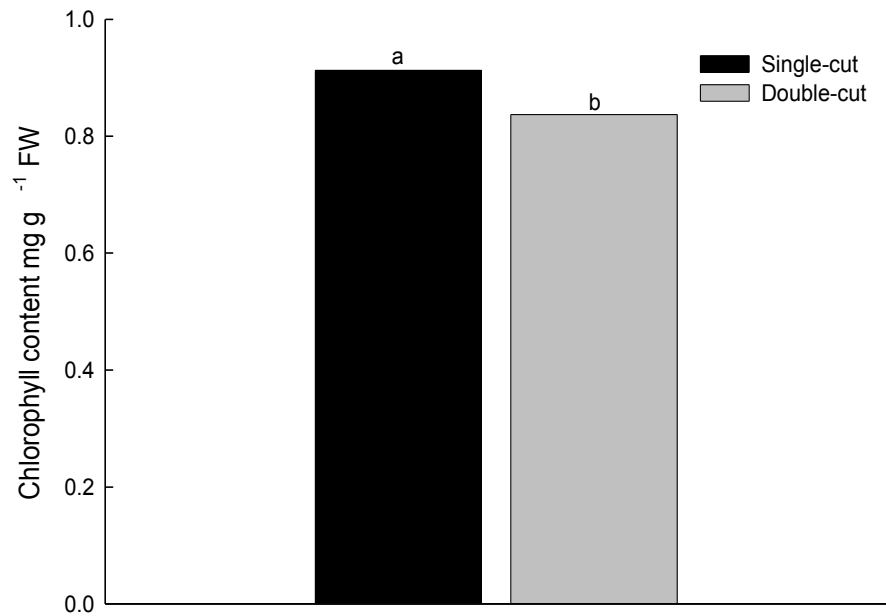
**Fig. 3.**  
Estimated rate  
of  
photochemistry  
of single- and  
double-cut 'L-

93' creeping bentgrass. Rate of photochemistry was estimated from  $F_m' - F/F_m'$  x photon flux density. Creeping bentgrass was clipped with mowers sharpened by using cylindrical grind, cylindrical with back grind, and carbide milling processes. Data averaged over area. Each data point is the mean of 180 observations. Means labeled with the same letter are not different at  $P \leq 0.05$ . Means were separated by using pairwise comparisons.



**Fig. 4.**  
Chlorophyll  
content of 'L-  
93' creeping  
bentgrass  
clipped with

mowers sharpened by using cylindrical grind, cylindrical with back grind, and carbide milling processes. Data averaged over frequency of cut and area. Each data point is the mean of 24 observations. Means labeled with the same letter are not different at  $P \leq 0.05$ . Means were separated by using pairwise comparisons.



**Fig. 5.**  
Chlorophyll  
content of  
single- and  
double-cut 'L-  
93' creeping

bentgrass. Data averaged over sharpening method and area. Each data point is the mean of 48 observations. Means labeled with the same letter are not different at  $P \leq 0.05$ . Means were separated by using pairwise comparisons.

## GENERAL CONCLUSION

The objectives of this research were to: 1) determine if double-cutting is more damaging to plant growth and development than single-cutting, 2) determine how frequently mowers should be sharpened to achieve the highest quality of cut. Mowing stress and injury were quantified by using measurements of activities of reactive oxygen species-scavenging enzymes, carbohydrate concentrations, chlorophyll content, estimated rate of photochemistry, leaf blade tissue damage, and lipid peroxidation, and efficiency of PSII.

Mowing damages leaf tissue, opening wounds that are susceptible to water loss and pests. Plants often increase formation of reactive oxygen species (ROS) in response to wounding (Orozco-Cardenas and Ryan, 1999). In our research, activities of catalase and ascorbate peroxidase were reduced in rolled, single-cut, and double-cut grasses. Reduced activities of these enzymes may result in accumulation of ROS in wounded grasses. However, no differences were observed in levels of lipid peroxidation among mowing treatments, indicating that accumulation of ROS was not sufficient to cause severe oxidative stress. Although toxic to macromolecules like carbohydrates, lipids, nucleic acids, and proteins at high concentrations, it has been suggested that ROS are a component of a signal transduction pathway that acclimates not-stressed tissue to potential stress (Karpinski et al., 1999).

Damage to photosystem II (PSII) often is a common manifestation of stress in wounded plants (Maxwell and Johnson, 2000). Decreases in efficiency of PSII were observed in rolled, single-cut, and double-cut grasses, indicating reduced efficiency of photosynthetic electron transport at PSII.

The duration and magnitude of changes observed in antioxidant enzyme activities and efficiency of PSII of double-cut grasses indicate that double-cutting is more injurious to plant growth than rolling or single-cutting.

It is necessary for grasses to regrow leaf tissue removed by mowing to develop the photosynthetic leaf area required for CO<sub>2</sub>-assimilation and energy production (Parsons et al., 1983). Products of fructan hydrolysis are used for respiration and to provide substrates for development of leaf and shoot tissue (Morvan-Bertrand et al., 1999; Donaghy and Fulkerson, 1998; Davidson and Milthorpe, 1966). In our research, levels of fructan were reduced in single- and double-cut grasses compared to rolled and not-cut grasses. Levels of fructan in single-cut grasses were similar to not-cut grasses 48 hours after mowing, whereas fructan levels of double-cut grasses were similar to not-cut grasses 60 hours after mowing. Glucose levels also were reduced in single- and double-cut grasses compared to rolled and not-cut grasses. Glucose levels were lowest in double-cut grasses compared to not-cut grasses.

Mowing results in transient decreases in the levels of fructan and glucose in creeping bentgrass. The duration of reduction in fructan levels was longest in double-cut grasses, indicating that double-cutting may limit the recuperative potential of creeping bentgrass.

Grasses cut with dull mowers are subjected to more stress than grasses mown with sharp mowers (Howieson and Christians, 2001). Regular mower sharpening is important to minimize mowing stress and achieve the best possible quality of cut. In our research, grasses cut with mowers sharpened by using the cylindrical grind method consistently had the largest leaf blade tissue damage and lowest chlorophyll content and estimated rate of photochemistry. Based on the results of this study, mowers sharpened by using the cylindrical grind method may need to be sharpened after every hectare of grass mown to maintain acceptable quality of cut. Based on leaf blade tissue damage, mowers sharpened by using cylindrical with back grind method may need to be re-sharpened after every 1.5 to 2.5 ha of grass mowed, whereas mowers sharpened by using the carbide milling method may need to be sharpened after every 3.5 ha of grass mowed. Double-cut grasses generally had

less leaf blade tissue damage compared to single-cut grasses, whereas estimated rates of photochemistry and chlorophyll content were reduced in double-cut grasses.

The results of this experiment suggest that without regular maintenance, mowers sharpened by using the cylindrical grind method can be more damaging to plant growth and development than mowers sharpened by using cylindrical with back grind and carbide milling methods. Double-cutting increases the severity of mowing stress compared to single-cutting, however double-cutting can improve the quality of cut of creeping bentgrass.

Development of a practical mowing program that balances agronomic requirements with sports play demands is important to produce dense, uniform, and visually appealing turf. Although double-cutting may improve playing surface uniformity and ball roll, caution should be used, especially during adverse environmental conditions, because grasses that have been double-cut may be less vigorous in growth and more susceptible to other stresses. If grasses must be double-cut, careful attention should be paid to water, fertility, and pest management because grasses will be less tolerant of environmental stresses.

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