Soluble Carbohydrates in Two Buffalograss Cultivars with Contrasting Freezing Tolerance

S. Ball, Y.L. Qian,¹ and C. Stushnoff

Department of Horticulture and Landscape Architecture, Colorado State University, Fort Collins, CO 80523-1173

ADDITIONAL INDEX WORDS. *Buchloe dactyloides*, cold hardiness, fructose, glucose, raffinose, sucrose, LT₅₀

ABSTRACT. No information is available regarding endogenous soluble carbohydrate accumulation in buffalograss [*Buchloe dactyloides* (Nutt.) Engelm.] during cold acclimation. The objective of this study was to determine composition of soluble carbohydrates and their relationship to freezing tolerance in two buffalograss cultivars, 609 and NE 91-118, with different freezing tolerances. The experiment was conducted under natural cold acclimation conditions in two consecutive years in Fort Collins, Colo. Based upon average LT₅₀ (subfreezing temperature resulting in 50% mortality) from seven sampling intervals in 1998–99 and six sampling intervals in 1999–2000, ‘NE 91-118’ survived 4.5 °C and 4.9 °C colder temperatures than ‘609’, during the 1998–1999 and 1999–2000 winter seasons, respectively. Glucose, fructose, sucrose, and raffinose were found in both cultivars in both years, and were generally higher in acclimated than pre- and post-acclimated stolons. Stachyose was not present in sufficient quantities for quantification. Cultivar NE 91-118 contained 63% to 77% more glucose and 41% to 51% more raffinose than ‘609’ in the 1998–99 and 1999–2000 winter seasons, respectively. In 1999–2000, fructose content in ‘NE 91-118’ was significantly higher than that of ‘609’. A significant negative correlation was found between LT₅₀ vs. all carbohydrates in 1999–2000, and LT₅₀ vs. sucrose and raffinose in 1998–99. Results suggest that soluble carbohydrates are important in freezing tolerance of buffalograss.

Buffalograss (*Buchloe dactyloides*), native to the shortgrass prairie region of North America, is a resource-efficient, warm-season grass with excellent drought and heat resistance (Beetle, 1950). With water becoming a more limited resource, and environmental issues of greater concern to the public, interest has increased in using more water- and resource-efficient buffalograss as turf (Riordan et al., 1993). Recently, a number of turf-type buffalograss cultivars have appeared on the market. These new cultivars provide improvements in turf color, density, texture, fall color retention, and spring greenup. However, because buffalograss is a warm season turfgrass, it is critical to examine freezing tolerance of each cultivar before recommendations are made for regional use. In a previous study, we examined freezing tolerance of six cultivars of buffalograss and found large intraspecific variation, with the lowest LT₅₀ values (subfreezing temperatures resulting in 50% mortality) ranging from –18.1 to –9.2 °C in 1 year and –21.6 to –14.0 °C the next (Qian et al., 2001). While these results are useful, our previous study did not provide information on physiological and biochemical mechanisms involved in buffalograss freezing tolerance.

Soluble carbohydrate content of many species has been found to be of importance in freezing tolerance. Soluble carbohydrate accumulation in stolons and/or rhizomes of several warm season turfgrasses is involved in the acquisition of freezing tolerance. In centipedegrass [*Eremochloa ophiuroides* (Munro) Hack], a positive correlation was observed between sucrose level and the number of surviving stolons in acclimated vs. nonacclimated stolons (Fry et al., 1993). Dunn and Nelson (1974) found that sucrose levels rose in stolons of three bermudagrass [*Cynodon dactylon* (L.) Pers.] cultivars during fall acclimation, though carbohydrate differences among cultivars were slight and not

related to winter survival. Some grass species, notably those with poor freezing tolerance such as St. Augustinegrass [*Stenotaphrum secundatum* (Walt.) Kuntze] and carpetgrass (*Axonopus affinis* Chase.), show no relationship between sucrose, total nonstructural carbohydrates, and freezing tolerance (Bush et al., 2000; Fry et al., 1991; Maier et al., 1994).

Most research on the relationships between freezing tolerance and carbohydrate content in warm season turfgrass species has focused on starch and sucrose (Bush et al., 2000; Dunn and Nelson, 1974; Fry et al., 1991; Maier et al., 1994; Rogers et al., 1975). Little is known about the presence and relationship of fructose, glucose, and galactose-containing oligosaccharides (such as raffinose and stachyose) to freezing tolerance in any turfgrass species. Hamman et al. (1996) found that endogenous levels of glucose, fructose, raffinose, and stachyose, but not sucrose, were strongly associated with cold hardiness in ‘Chardonnay’ and ‘Riesling’ grapevines (*Vitis vinifera* L.). In a study with six woody plant species, only galactose-containing raffinose and stachyose were strongly related to cold hardiness (Stushnoff et al., 1993). In aspen (*Populus tremuloides* Michx.), endogenous raffinose and stachyose increased as temperatures decreased in early winter and diminished as temperatures rose in spring (Cox and Stushnoff, 2001). Recent studies with alfalfa (*Medicago sativa* L.) (Castonguay et al., 1995), honeysuckle (*Lonicera caerulea* L.) (Imanishi et al., 1998), and coniferous trees (Hinesley et al., 1992) indicated that sucrose alone is not likely to confer tolerance to low temperature and that raffinose and stachyose play determinant roles in low temperature tolerance. Furthermore, incubation in the presence of raffinose and fructose enhanced freezing tolerance of Eucalyptus (*Eucalyptus gunnii* Hook.) cells (Leborgne et al., 1995).

The buffalograss cultivars NE 91-118 and 609 were developed and released from the University of Nebraska (Riordan et al., 1992). Both are tetraploids and homozygous vegetatively propagated cultivars (Johnson et al., 1998). In an earlier study, we found that NE 91-118 suffered substantially less freezing injury during Colorado winters than 609 (Qian et al., 2001). Comparing soluble carbohydrate composition and content of buffalograss

Received for publication 20 April 2001. Accepted for Publication 3 Oct. 2001. Funding was provided by the Rocky Mountain Turfgrass Research Foundation and Colorado Agricultural Experimental Station (project 780). The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

¹Corresponding author; e-mail yaqian@lamar.colostate.edu.

cultivars having different freezing tolerance may aid in elucidating the freezing tolerance mechanisms in buffalograss. Therefore, the objectives of this study were to: examine seasonal changes in soluble carbohydrate content (sucrose, glucose, fructose, raffinose, and stachyose) in two cultivars of buffalograss, 609 and NE 91-118, and determine possible relationships among soluble carbohydrate content and freezing tolerance in buffalograss.

Materials and Methods

CULTIVAR ESTABLISHMENT. Plugs of '609' and 'NE 91-118' buffalograss were planted in six 3 × 3-m plots at the W.D. Holley Plant Environmental Research Center, Colorado State University, Fort Collins, in July 1996. Each cultivar was replicated three times in a randomized complete block design. Soil was a Nunn clay loam (fine, smectitic, mesic Aridic Argiustolls). During the 1998 and 2000 growing seasons, plots were fertilized every June and August with a 40N-0P-0K fertilizer with N at 49 kg·ha⁻¹. Daily maximum and minimum temperatures were recorded from a weather station located within 2 km of the study site (Fig. 1). During growing season, grasses were mowed weekly at 7.0 cm.

FREEZING TEST TO DETERMINE LETHAL LOW TEMPERATURE. Freezing tests were conducted at approximately monthly intervals from Oct. 1998 through Apr. 1999 and from Oct. 1999 to May 2000. Detailed procedures to determine lethal low temperature, defined as the subfreezing temperature that resulted in 50% mortality (LT₅₀), were described in a previous publication (Qian et al., 2001). Briefly, stolons were sampled from field plots, and divided into 8 to 12 fractions. Each fraction, containing at least 10 stolons, was subjected to a low temperature treatment using a thermocontrolled freezer (Tenny Jr. Programmable Freezer, Tenny, Inc., South Brunswick, N.J.). The freezing chamber was programmed to cool linearly at 2 °C/h after 16 h at 0 °C. Stolons were removed as target temperatures were reached. Samples were thawed overnight at 2 °C following removal from the freezing chamber. Nonfrozen controls were held at 2 °C.

Following thawing, individual nodes were planted in a plastic cone (3 cm in diameter, 8 cm deep) filled with commercial potting soil. All plants were maintained in the greenhouse at 32 °C/25 °C (day/night) under natural greenhouse light conditions. Irrigation was applied by a mist system to provide 3 to 5 mm·d⁻¹. Stolon survival percentage was recorded by observing regeneration of shoots 2 to 4 weeks after planting.

CARBOHYDRATE ANALYSIS. On the same dates that freezing tests were conducted, additional stolon samples were collected from each plot. Samples were freeze-dried immediately (Genesis 25LL lyophilizer; Virtis, Gardiner, N.Y.), and subsequently stored in airtight vials at -20 °C before and after grinding using a Wiley Mill (Arthur Thomas Co., Philadelphia). Samples were then sieved through a 100-mesh (0.254-mm) screen. About 1 mg of screened sample was weighed and placed into a test tube containing 50 mL of methyl glucopyranoside at 1 mg·mL⁻¹ as an internal standard. Carbohydrate derivitization was carried out according to Cox and Stushnoff (2001) and Sweeley et al. (1963), by adding 400 mL pyridine, 80 mL hexamethyldisilazane, and 40 mL trimethylchlorosilane. Tubes were capped tightly, heated at 80 °C for 20 min,

then vials were dried under a stream of air. Hexane was added to each tube, and the supernatant was transferred to a clean tube and dried under an air stream. Finally, the dried, derivitized samples were dissolved in 200 mL hexane before injection into the gas chromatograph. As a derivitization control, a standard solution containing 25 mL (1 mg·mL⁻¹) each of fructose, sucrose, glucose, raffinose, stachyose, and the internal standard was included and derivitized along with each set of eight samples.

Derivitized samples in hexane (1 mL) were injected into a gas chromatograph (HP5890 Series II; Hewlett Packard, Boulder, Colo.) with a 30-m silica capillary column (DB-1, 0.25-mm inner diameter, 0.25-mm film thickness) (J&W Scientific, Folsom, Calif.) and a flame ionization detector. Helium was the carrier gas at a flow rate of 2 mL·min⁻¹. Carbohydrates present in the samples were identified by comparing retention times with known standards. Carbohydrate quantifications were determined by comparing peak areas to internal standard area using Peak Simple 1.72 (SRI, Inc., Torrance, Calif.).

DATA ANALYSIS. Data were subjected to analysis of variance (ANOVA) to test effects of cultivar, sampling time, and their interactions using GLM procedures of SAS (SAS Inst., Inc. 1991). Two seasons of freezing tolerance and carbohydrate data were analyzed and presented separately because of a significant year effect. When appropriate, means were separated by Fisher's protected least square difference (LSD) at *P* = 0.05 or otherwise noted. Pearson's correlation coefficient between individual carbohydrates and lethal low temperature (LT₅₀) were analyzed using the CORR procedure of SAS (SAS Inst., Inc. 1991) for each year.

Results and Discussion

FREEZING TOLERANCE. Minimum and maximum air temperature declined steadily from September to January with minimum air temperature falling well below 0 °C between November and February and rising above 0 °C from March to May (Fig. 1). Data on LT₅₀, which represents the temperature at which 50% of nodes sampled were killed, demonstrated that the freezing tolerance of each cultivar followed a typical seasonal pattern in both years (Fig. 2).

In 1998–99, cultivar 609 increased its freezing tolerance in fall, reaching a maximal freezing tolerance between January and February with deacclimation occurring in March. The freezing

Fig. 1. Minimum and maximum air temperatures in Fort Collins, Colo., from (A) September to April 1998–99 and (B) 1999–2000. Arrows indicate stolon sampling dates.

1998 -1999

1999-2000

Fig. 2. Freezing tolerance, expressed as LT_{50} , of 'NE 91-118' and '609' buffalograsses in (A) 1998–99 and (B) 1999–2000. Lowercase letters are mean ($n = 3$) separation among sampling dates for '609' and uppercase letters are mean separation among sampling dates for 'NE-91-118', $P < 0.05$.

tolerance of 'NE 91-118' increased greatly from October to November, reaching its maximum in February and March. Deacclimation occurred in April for 'NE 91-118'.

In 1999–2000, '609' increased its freezing tolerance from October to January, reaching the lowest LT_{50} of -18.0°C in January. Deacclimation occurred in March. 'NE-91-118' had a LT_{50} of -14.0°C in October, suggesting some acclimation had occurred before October sampling. 'NE-91-118' exhibited additional 7.0°C acclimation from October to November, retained its maximal freezing tolerance between November and January, and deacclimation occurred between March and April.

On all but two testing dates (17 Oct. 1998 when plants were nonacclimated, and 2 May 2000 when plants were completely deacclimated), 'NE 91-118' exhibited greater freezing tolerance than '609'. The mean LT_{50} value of 'NE 91-118' was 4.5°C and 4.9°C colder than that of '609', during 1998–99 and 1999–2000 winter seasons, respectively.

SOLUBLE CARBOHYDRATES. Stachyose was not present in large enough amounts for quantification in either cultivar. Fructose, glucose, sucrose, and raffinose were present in both cultivars in both years, with sucrose and glucose being the most predominant sugars, representing $\approx 75\%$ of the total soluble sugar tested. Raffinose was present in the lowest quantities, only representing about 1% of the total soluble sugar tested (Fig. 3). Concentrations of all soluble carbohydrates varied significantly with sampling time (Table 1).

1998 to 1999. In the 1998–99 winter season, fructose and glucose content of '609' did not show clear trends of seasonal change in their concentrations. Fructose and glucose content in 'NE 91-118', however, exhibited trends of increase from October to December, reaching maximum concentrations of $21.1 \mu\text{mol}\cdot\text{g}^{-1}$ dry weight (DW) for fructose and $32.5 \mu\text{mol}\cdot\text{g}^{-1}$ DW for glucose in December, subsequently declining from December to minimum concentrations of 2.6

$\mu\text{mol}\cdot\text{g}^{-1}$ DW for fructose and $3.5 \mu\text{mol}\cdot\text{g}^{-1}$ DW for glucose in April. From October to December, the concentration of sucrose increased about 155% for both 'NE 91-118' and '609' while the concentration of raffinose increased 232% for 'NE 91-118' and 173% for '609'. Reduction of sucrose and raffinose occurred after December, reaching the lowest concentration in March or April. The increase of soluble carbohydrates during fall acclimation is higher in buffalograss compared to other warm season turfgrass species (Fry et al., 1993; Rogers et al., 1975). Rogers et al. (1975) observed a 48% increase in total nonstructural carbohydrates in Meyer zoysiagrass (*Zoysia japonica* Steud.) during fall acclimation. Fry et al. (1993) observed that centipedegrass stolon sucrose content increased 47% during acclimation in

a controlled environment, and was positively correlated with stolon survival. Despite different analytical procedures used in the aforementioned studies, the much greater rate of increase in soluble carbohydrates during cold acclimation in buffalograss observed in the present investigation may have contributed to the

Fig. 3. Mean concentrations of fructose, glucose, sucrose, and raffinose of 'NE 91-118' and '609' buffalograsses from October 1998 to April 1999 (left panel) and from October 1999 to May 2000 (right panel). Each symbol represents the mean of three observations. Vertical bars represent SE.

Table 1. ANOVA with mean squares and treatment significance of LT₅₀, fructose, glucose, sucrose, and raffinose of 'NE-91-118' and '609' buffalograsses from September to April 1998–99 and 1999–2000 winter seasons in Fort Collins, Colo.

Source	Mean square				
	LT ₅₀	Fructose	Glucose	Sucrose	Raffinose
1998–99					
Cultivar (C)	194.0****	20.9 ^{NS}	312.3*	38.2 ^{NS}	0.45*
Block (B)	6.2*	10.9 ^{NS}	12.8 ^{NS}	5.2 ^{NS}	0.1 ^{NS}
C × B	7.9*	45.6 ^{NS}	28.2 ^{NS}	3.2 ^{NS}	0.002 ^{NS}
Month (M)	86.1****	124.4**	137.8*	622.7***	0.56*
C × M	12.4**	22.2 ^{NS}	29.9 ^{NS}	56.7 ^{NS}	0.07 ^{NS}
1999–2000					
Cultivar (C)	215.0***	66.2*	2017.0***	13.3 ^{NS}	0.86*
Block (B)	1.0 ^{NS}	28.1 ^{NS}	26.8 ^{NS}	26.4 ^{NS}	0.15 ^{NS}
C × B	0.3 ^{NS}	19.8 ^{NS}	16.2 ^{NS}	5.3 ^{NS}	0.10 ^{NS}
Month (M)	141.0***	328****	1767.0***	799.0***	1.1*
C × M	5.5 ^{NS}	19.8 ^{NS}	173.2 ^{NS}	5.8 ^{NS}	0.1 ^{NS}

NS,*,**,***,**** Nonsignificant or significant at $P \leq 0.1$, 0.01, 0.001, and 0.0001, respectively.

greater freezing tolerance of buffalograss in comparison with zoysiagrass, centipedegrass, and bermudagrass (Qian et al., 2001).

ANOVA also indicated a cultivar effect on glucose and raffinose content ($P < 0.1$) (Table 1). 'NE 91-118' contained 63% more glucose and 41% more raffinose than '609' during the 1998–99 winter season.

1999 to 2000. In the 1999–2000 winter season, concentrations of fructose, glucose, sucrose, and raffinose, changed markedly with the season. Glucose and fructose content in stolons of 'NE 91-118' increased 84% and 147% from October to November, remained at maximum levels of $\approx 24.5 \mu\text{mol}\cdot\text{g}^{-1}$ DW for fructose and $\approx 56.0 \mu\text{mol}\cdot\text{g}^{-1}$ DW for glucose from November to January, and declined thereafter to $5.9 \mu\text{mol}\cdot\text{g}^{-1}$ DW of fructose and $7.3 \mu\text{mol}\cdot\text{g}^{-1}$ DW of glucose in April. The seasonal patterns of glucose and fructose of '609' were similar to 'NE 91-118', except they reached their maximal contents in November–December and declined quickly thereafter. Sucrose and raffinose concentrations in '609' and 'NE 91-118' increased from October to January, reached their maximum of 44.3 to $46.5 \mu\text{mol}\cdot\text{g}^{-1}$ DW for sucrose and 1.15 to $1.80 \mu\text{mol}\cdot\text{g}^{-1}$ DW for raffinose in January, and declined thereafter.

During the winter season of 1999–2000, the average fructose, glucose, and raffinose concentrations over the season were 17.5, 38.9, and $0.95 \mu\text{mol}\cdot\text{g}^{-1}$ DW, respectively, in 'NE 91-118'. These concentrations were 23.5%, 77.0%, and 51.1% higher, respectively, than those of '609'. We did not find a significant effect of cultivar on sucrose. Our results agree with Dunn and Nelson (1974), who found that sucrose levels rose in stolons of three bermudagrass cultivars during fall acclimation, but differences in sucrose levels among cultivars were slight and not related to winter survival. Sucrose has also been found not to be associated with cold hardiness in several woody trees and in grape vines (Stushnoff et al., 1993).

CORRELATION OF CARBOHYDRATE CONTENT AND FREEZING TOLERANCE. In 1998–99, glucose and fructose were not correlated with freezing tolerance (Table 2). In 1999–2000, however, correlations between LT₅₀ vs. fructose and glucose were significant with linear correlation coefficients of -0.62 and -0.77 , respectively. Sucrose and raffinose contents were negatively correlated with the LT₅₀ value in both years with correlation coefficients of -0.53 to -0.72 for sucrose and -0.44 to -0.50 for raffinose for 1998–99 and 1999–2000, respectively. This suggests that the higher the sucrose and raffinose contents, the more cold tolerant

the buffalograss (Table 2). Nevertheless, the two cultivars with contrasting freezing tolerance did not differ appreciably with respect to accumulation of sucrose, but differed in glucose and raffinose accumulations in both years, and in fructose content during the 1999–2000 winter season (Table 1). Compared to '609', 'NE 91-118' exhibited better freezing tolerance, higher mean glucose and raffinose content in both years, and higher fructose content in 1999–2000 (Figs. 2 and 3). These results suggest that, despite accumulation of high concentrations of sucrose in response to the change of environmental temperature, the pattern of accumulation does not indicate a direct relationship to freezing tolerance between different cultivars. The concentration of glucose and raffinose, along with fructose, may be important factors which contributed to the greater freezing tolerance of 'NE 91-118' than that of '609', despite low correlation coefficients observed (0.44 to 0.77).

Correlation analysis also indicated that fructose and glucose were highly interrelated ($r = 0.82$ to 0.88), showing similar seasonal fluctuation patterns. Likewise sucrose and raffinose shared strikingly similar seasonal trends with correlation coefficients of 0.84 to 0.81. These similarities may be related to the shared metabolic pathway during synthesis. Raffinose is synthesized by addition of a galactosyl unit to sucrose (Avigad and Dey, 1997).

Table 2. Correlation coefficients between freezing tolerance (LT₅₀) and individual soluble carbohydrate contents in stolons of '609' and 'NE 91-118' buffalograsses sampled from field plots from September to April 1998–99 and 1999–2000. A negative coefficient indicates that greater freezing tolerance (lower LT₅₀) was related to an increase in carbohydrate content.

Parameter	Fructose	Glucose	Sucrose	Raffinose
1998–99				
LT ₅₀	-0.21^{NS}	-0.24^{NS}	-0.53^*	-0.44^*
Fructose		0.82^{**}	0.33^{NS}	0.21^{NS}
Glucose			0.51^*	0.44^*
Sucrose				0.81^{**}
1999–2000				
LT ₅₀	-0.62^{**}	-0.77^{**}	-0.72^{**}	-0.50^{**}
Fructose		0.88^{**}	0.47^*	0.25^{NS}
Glucose			0.65^{**}	0.50^*
Sucrose				0.84^{**}

NS,*,** Nonsignificant or significant at $P \leq 0.01$ and 0.0001, respectively.

The present investigation has documented that in buffalograss higher concentrations of endogenous soluble carbohydrates were accumulated in December during the 1998–99 winter season and in November–January during 1999–2000 than during preacclimation and post acclimation periods. This suggests that the acclimation process involves production of soluble carbohydrates as cold hardiness develops, and their degradation as hardiness diminishes during spring. Soluble carbohydrates may enhance freezing tolerance by reducing the freezing point, serving as a cryoprotectant, osmotic buffer, and/or through stabilization of proteins and phospholipids. The fact that we found higher raffinose contents in cold hardy ‘NE 91-118’ than in cold tender ‘609’ suggests that, despite its low content, raffinose may play a significant role in enhancing freezing tolerance in buffalograss. Koster and Leopold (1988) and Caffrey et al. (1988) suggested that raffinose and other galactose-containing oligosaccharides might confer desiccation tolerance by preventing crystallization of sucrose, thereby facilitating availability of sucrose during the drying of seeds. It is possible that raffinose plays a role in freezing tolerance of buffalograss by protection against freeze-induced cell dehydration.

Literature Cited

- Avigad, G. and P.M. Dey. 1997. Carbohydrate metabolism: Storage carbohydrates, p. 143–204. In: P.M. Dey and J.B. Harborne (eds.). Plant biochemistry. Academic Press. San Diego.
- Beetle, A.A. 1950. Buffalograss-native of the shortgrass plains. Univ. Wyo. Agr. Expt. Sta. Bul. 293.
- Bush, E., P. Wilson, D. Shepard, and J. McCrimmon. 2000. Freezing tolerance and nonstructural carbohydrate composition of carpetgrass (*Axonopus affinis* Chase). HortScience 35:187–189.
- Caffrey, M., V. Fonseca, and A.C. Leopold. 1988. Lipid–sugar interactions: Relevance to anhydrous biology. Plant Physiol. 86:754–758.
- Castonguay Y., P. Nadeau, P. Lechasseur, and L. Chouinard. 1995. Differential accumulation of carbohydrates in alfalfa cultivars of contrasting winter hardiness. Crop Sci. 35:509–516.
- Cox, S.E. and C. Stushnoff. 2001. Temperature-related shifts in soluble carbohydrate content during dormancy and cold acclimation in *Populus tremuloides*. Can. J. For. Res. 31:730–737.
- Dunn, J.H. and C.J. Nelson. 1974. Chemical changes occurring in three bermudagrass turf cultivars in relation to cold hardiness. Agron. J. 66:28–31.
- Fry, J.D., N.S. Lang, and R.G.P. Clifton. 1991. Freezing resistance and carbohydrate composition of ‘Floratum’ St. Augustinegrass. Hort-Science 26:1537–1539.
- Fry, J.D., N.S. Lang, R.G.P. Clifton, and F.P. Maier. 1993. Freezing tolerance and carbohydrate content of low-temperature-acclimated and non-acclimated centipedegrass. Crop Sci. 33:1051–1055.
- Hamman, Jr., R.A., I.E. Dami, T.M. Walsh, and C. Stushnoff. 1996. Seasonal carbohydrate changes and cold hardiness of Chardonnay and Riesling grapevines. Amer. J. Enol. Viticult. 47:31–36.
- Hinesley, L.E., D.M. Pharr, L.K. Snelling, and S.R. Funderburk. 1992. Foliar raffinose and sucrose in four conifer species: Relationship to seasonal temperature. J. Amer. Soc. Hort. Sci. 117:852–855.
- Imanishi, H.T., T. Suzuki, K. Masuda, and T. Harada. 1998. Accumulation of raffinose and stachyose in shoot apices of *Lonicera caerulea* L. during cold acclimation. Scientia Hort. 72:255–263.
- Johnson, P.G., T.P. Riordan, and K. Arumuganathan. 1998. Ploidy level determinations in buffalograss clones and populations. Crop Sci. 38:478–482.
- Koster, K.L. and A.C. Leopold. 1988. Sugars and desiccation tolerance. Plant Physiol. 88:829–832.
- Leborgne, N., C. Teulieres, S. Travert, M. Rols, J. Teissie, and A.M. Boudet. 1995. Introduction of specific carbohydrates into *Eucalyptus gunnii* cells increases their freezing tolerance. Euro. J. Biochem. 229:710–717.
- Maier, F.P., N.S. Lang, and J.D. Fry. 1994. Freezing tolerance of three St. Augustinegrass cultivars as affected by stolon carbohydrate and water content. J. Amer. Soc. Hort. Sci. 119:473–476.
- Qian, Y.L., S. Ball, Z. Tan, A.J. Koski, and S.J. Wilhelm. 2001. Low temperature tolerance of six cultivars of buffalograss. Crop Sci. 41:1174–1178.
- Riordan, T.P., S.A. Shazer, F.P. Baxendale, and M.C. Engelke. 1992. Registration of ‘609’ buffalograss. Crop Sci. 32:1511.
- Riordan, T.P., S.A. Shazer, J.M. Johnson-Cicalese, and R.C. Shearman. 1993. An overview of breeding and development of buffalograss for golf course turf. Intl. Turfgrass Soc. Res. J. 7: 816–822.
- Rogers, R.A., J.H. Dunn, and C.J. Nelson. 1975. Cold hardening and carbohydrate composition of ‘Meyer’ zoysia. Agron. J. 67: 836–838.
- SAS Institute, Inc. 1991. SAS/STAT user’s guide. SAS Inst., Inc., Cary, N.C.
- Stushnoff, C., R.L. Remmele, Jr., V. Essensee, and M. McNeil. 1993. Low temperature induced biochemical mechanisms: Implications for cold acclimation and de-acclimation, p. 647–657. In: M.B. Jackson and C.R. Black (eds.). Interacting stresses on plants in a changing climate. vol. 116. NATO ASI Ser. Springer-Verlag, Berlin.
- Sweeley, C.C., R. Bentley, M. Makita, and W.W. Wells. 1963. Gas–liquid chromatography of trimethylsilyl derivatives of sugars and related substances. J. Amer. Chem. Soc. 85:2497–2507.