Thermochimica Acta 479 (2008) 7–11

Contents lists available at ScienceDirect

Thermochimica Acta

journal homepage: www.elsevier.com/locate/tca

Predicting relative low-temperature tolerance of non-acclimatized buffalograss using calorimetric data

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ARTICLE INFO

Article history:
Received 9 November 2007
Received in revised form 3 September 2008
Accepted 4 September 2008
Available online 11 September 2008

Keywords:
Calorimetry
Freezing tolerance
Low-temperature tolerance
Buchloe

ABSTRACT

No information is available regarding the usefulness of calorimetry in assessing freeze tolerance, cold hardiness and associated mechanisms in turfgrass. In this study, isothermal and scanning microcalorimetric techniques were used to determine relative low-temperature tolerance and freezing points of leaf and root segments of three, non-acclimatized buffalograss (*Buchloe dactyloides* (Nutt.) Engelm.) genotypes. Based on isothermal microcalorimetric data, genotype NE91-118 (cultivar now known as 'Prestige') was less affected by low temperatures than either genotype 609 or UCD 95 in the −5 to −15 °C range suggesting greater cold hardiness for 'Prestige'. Among the three genotypes tested, leaf and root segments of 'Prestige' froze at the lowest temperature, 609 was intermediate, and UCD 95 froze at the highest temperature. Scanning microcalorimetry showed that the freezing points of root segments were 7.5–8.8 °C higher than leaf segments for all three genotypes. Our results suggested that the lower freezing temperatures and the greater ability to maintain metabolic stability after exposure to low temperatures contributed to the higher degree of freezing tolerance of 'Prestige'. Results indicated that calorimetry was an effective tool for determining low-temperature tolerance.

1. Introduction

Low-temperature tolerance of warm-season turfgrasses has been the focus of intensive research for many years [6]. However, few studies have been published that determined the low-temperature tolerance of warm-season turfgrasses [9,12,14,16,21]. Thermal injury is a common problem in many areas of the world. Injury threshold temperatures depend on the species and can occur at both high and low temperatures. A complex relationship exists between metabolic activity, temperature and time of exposure to temperature extremes [2]. Research to explain low-temperature tolerance in relation to biochemical characteristics of warm-season turfgrass tissues has been done [1,4]. This work has shown correlations between low-temperature tolerance and the composition of soluble carbohydrates in turfgrasses. Ball et al. [1] found that soluble carbohydrates are important in freezing tolerance of buffalograss.

Calorimetry has proven its usefulness in biological applications for many years. It is an effective tool for measuring metabolic heat production rates of organisms, which can be used as an indicator of growth rates, effects of environmental stresses, and of the biochemical pathways used by the organism. Since the early 1990s, calorimetry technologies and methodologies have rapidly made valuable contributions in understanding basic plant physiology and have demonstrated many practical applications. One method to examine the ability of plants to recover from low and high temperature stresses was developed by Rank et al. [19]. Cell and leaf tissues in the calorimeter were rapidly cycled between a reference temperature in the mid-range of thermal stability, a selected low or high stress temperature, and back to the reference temperature, to measure amounts of recovered metabolic activities. They cycled between different temperatures and for different lengths of time to develop a surface plot of recovered metabolic activity with varying temperatures and time of exposure at each temperature.

Freezing water releases heat and, at sub-zero temperatures, the low-temperature exotherm (LTE) can be detected using differential scanning calorimeter and differential thermal analysis (DTA) [15]. In some species an LTE is not observed until temperatures below the killing point are reached. This may be due to supercooling (whereby the water is prevented from freezing at temperatures far below 0 °C) [8]. Supercooling is one avoidance mechanism

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doi:10.1016/j.tca.2008.09.002

non-acclimatized plants use to avoid freezing injury [20]. It along with subsequent LTE has been associated with northern distribution limits for the production of two fruit species in North America [17] and for evaluation of *Pyrus* cold hardiness [13,18]. If plants are exposed to low, non-freezing temperatures over a period of days or weeks, they may increase their freezing tolerance. This process is known as cold acclimation or cold hardening [11].

There are two main methods of using calorimetry for answering different physiological questions. The first is scanning calorimetry, where tissues are scanned over varying temperatures. This technique is useful for discovering plant responses to different temperatures, defining temperature sensitivities and for the detection of LTE as water freezes [10]. This particular application of the differential scanning microcalorimeter is a recent improvement of the DTA techniques used in the past to investigate what role(s) supercooling and/or increased tolerance to freezing play in plants' survival of low temperatures [3,7].

The second method is isothermal calorimetry which can be used to examine total metabolic heat production and growth rates of plant samples and how these rates change with different artificial or natural factors. This information can be useful in the selection of plants with desirable metabolic and growth characteristics to specific environments [5].

Buffalograss is a warm-season turfgrass species native to the Great Plains of North America distributed from Mexico to Canada and from the eastern slope of the Rocky Mountains to the Mississippi River. An approach using isothermal and/or scanning microcalorimetric techniques could be a useful and rapid way of determining low-temperature tolerance of breeding lines of buffalograss and other warm-season turfgrasses. In the present study isothermal and scanning microcalorimetric techniques were employed to determine relative thresholds of low-temperature tolerance to freezing of three buffalograss (*Buchloe dactyloides* (Nutt.) Engelm.) genotypes.

2. Materials and methods

Plugs (10.8 cm diameter and ~5 cm deep) of '609', 'Prestige' and 'UCD 95' buffalograss were sampled from field plots in Fort Collins, CO when buffalograss was still in winter dormancy. One plug of each genotype was planted in a 9-l plastic container filled with UC mix (by volume, 42% composted fir bark, 33% peat moss and 25% sand) and kept in a greenhouse ($28 \pm 4\,^{\circ}C$) under natural daylength conditions for the duration of the experimentation (April to July in 2002). The developing sod was watered daily and fertilized with a 10-10-10 NPK fertilizer twice per month which provided ~20 mg N/container/fertilization. When the sod was fully developed, stolons and leaf segments were sampled for calorimetric measurements. In March, 2005, new plugs of the three genotypes were planted as above and the calorimetric measurements were repeated from April to June, 2005. An analysis of variance was conducted on the two sets of data using PROC GLM (SAS Institute, Inc., Cary, NC); there were no significant differences so the data from the two time periods were pooled. For both sets of experiments, leaf and root samples were taken from actively growing plants forming stolons that extended over the edges of their containers.

Calorimetric measurements were made with a CSC (Calorimetry Sciences Corporation, Provo, UT) Model 4100 differential scanning calorimeter operated in either isothermal or scanning mode. The calorimeter had four removable ampules, three of which were used for simultaneous measurements of rates of metabolic heat production (q, $\mu J\,s^{-1}$) with the remaining empty ampule used as a reference. Ampules were thin–walled cylinders of 1-cm^3 volume constructed of Hastelloy C with a screw cap sealed with a Viton gasket.

2.1. Isothermal microcalorimetry

Stolon tips (approximately 1-cm long) from each buffalograss genotype were placed, one per ampule, in each of the three ampules. The ampules were closed, placed in the calorimeter that had been programmed to make readings of q at 20 °C following 1-h exposures to the following temperatures: 0, −2.5, −5, −7.5, −10, −12.5 and −15 °C. The sequence was as follows: an initial measurement at 20 °C was made immediately after the stolon tips were placed in the calorimeter. The calorimeter scanned down (scandown rate = $120\,^{\circ}C\,h^{-1}$) to 0 °C for 1-h followed by a scan back up (scanup rate = $120\,^{\circ}C\,h^{-1}$) to 20 °C where a second measurement was made. The calorimeter then scanned down to −2.5 °C for 1-h followed by a second scan back up to 20 °C for a third measurement. This process was followed five more times for exposures to −5, −7.5, −10, −12.5 and −15 °C all followed by measurements of q at 20 °C. This sequence with the three stolon tips was repeated two more times for each of the three buffalograss ampules resulting in nine replicates for each genotype at each temperature. The data were plotted using the initial q measurement at 20 °C as the basis for comparing all other readings. The metabolic heat rates of those measurements, serving as an indication of metabolic activity, were expressed as a percent of the initial q taken at 20 °C (control). The genotypes' responses to low-temperature were assessed by fitting the data to quadratic equations (SAS Institute, Inc., Cary, NC). Those equations were used to compare the three genotypes and to make estimations of their relative loss of metabolic activity at varying low temperatures.

2.2. Scanning microcalorimetry

Ten, 1-cm-long leaf segments from each buffalograss genotype were placed in one calorimeter ampule. Leaf segments were obtained by taking 10 leaves (5–10 cm long) of each genotype, collating them and cutting them horizontally twice, 1 cm apart. The resulting 1-cm-long leaf segment was taken from approximately the middle of the leaf. The Model 4100 calorimeter could measure three ampules simultaneously so all three genotypes were measured at the same time. The initial calorimeter temperature was set to 2 °C. After 10 min the calorimeter was programmed to begin a slow ($1\,^{\circ}C\,h^{-1}$) scan downward from 2 °C to −25 °C. Heat (approximately 500–750 $\mu J\,s^{-1}$) was given off when individual leaf segments froze. This procedure was repeated resulting in freezing data from 20 leaf segments from each genotype. The temperature at which each leaf segment froze was determined from the plotted calorimeter data and means for each genotype were calculated. A similar procedure was followed with root segments from the three genotypes. Root segments were obtained from 1- to 3-cm-long stolon tips that had been placed on blotter paper saturated with deionized water in 112 mm × 112 mm × 40 mm square, plastic enclosures. After 10–20 days roots that had emerged and elongated from the stolons, 3–7 root segments (1-cm long) from each genotype were taken and placed in the calorimeter ampules for measurements. This procedure with root segments was repeated until freezing data from 15, 24 and 24 root segments were obtained from genotypes 'Prestige', 609 and UCD 95, respectively. The heat generated upon freezing of the each root segment was approximately 2000–3000 $\mu J\,s^{-1}$. This was more heat than was generated from leaf segments because the root segments contained more water. Data from successive scans were pooled and statistically analyzed using the General Linear Model procedure in SAS.

2.3. Genotype freeze testing—determination of LT_{50}

Stolons of buffalograss genotypes 'Prestige', 609 and UCD 95 were sampled on 1–2 month intervals from October to May in Fort Collins, CO. On each sampling date, stolons were collected from each of three replicated plots for each genotype. After washing, stolons were divided into 9–10 fractions. Each fraction, which contained at least 10 nodes, was individually wrapped in moist tissues and then placed in aluminum foil for a targeted freezing temperature. Samples were subjected to low-temperature treatments using a thermo-controlled freezer (Tenny Jr. Programmable Freezer, Tenny Inc., South Brunswick, NJ). The freezing chamber was programmed to cool linearly at 2 °C/h after an initial 16 h at −2 °C. One fraction of stolons was removed at each target temperature. Target temperatures ranged from −4 to −26 °C at −2 °C intervals varied with sampling dates, air temperatures, and expected acclimation to cover the range of expected 50% lethal temperatures.

Samples were thawed overnight at 2 °C as soon as they were removed from the freezing chamber. Non-frozen controls were kept at 2 °C during the freezing treatment. Following thawing, individual nodes were planted in foam plug trays with each square hole measuring 3 cm × 3 cm × 8 cm filled with a commercial potting soil medium. All plants were maintained in the greenhouse at approximately 25 °C. Irrigation was applied by a mist system to provide about 3–5 mm/day. Stolon survival was recorded by observing regeneration of shoots 3–4 weeks after planting. LT_{50} values were determined for each replications of each cultivar. Means and standard deviations were calculated to compare the response of the three genotypes.

3. Results

3.1. Isothermal microcalorimetry

Exposure to temperatures between 0 and −5 °C had the same effect on all three buffalograss genotypes (Fig. 1).

However, after a 1-h exposure to −7.5 °C the activity (expresses as % of control) of UCD 95 was significantly lower than that of 'Prestige'. After a 1-h exposure to −10 °C both UCD 95 and 609 had significantly lower metabolic activities than 'Prestige'. This difference increased with decreasing temperature exposures of −12.5 and −15 °C. Another way of assessing the response of the three genotypes to low-temperature exposure was to determine, using the fitted quadratic equations, the percent loss of metabolic activity after exposure to low temperatures relative to the initial activity at 20 °C for each of the genotypes. When this was done, it was determined that there was a 25% loss in metabolic activity at −4.6, −3.2 and −2.1 °C for 'Prestige', 609 and UCD 95, respectively, a 50% loss in metabolic activity at −10.4, −7.6 and −7.9 °C for 'Prestige', 609 and UCD 95, respectively and a 75% loss in metabolic activity at −14.1, −11.0 and −11.5 °C for 'Prestige', 609 and UCD 95, respectively. Therefore, the temperature necessary to cause activity loss of 25, 50 or 75% was always lower for 'Prestige' than for 609 and UCD 95.

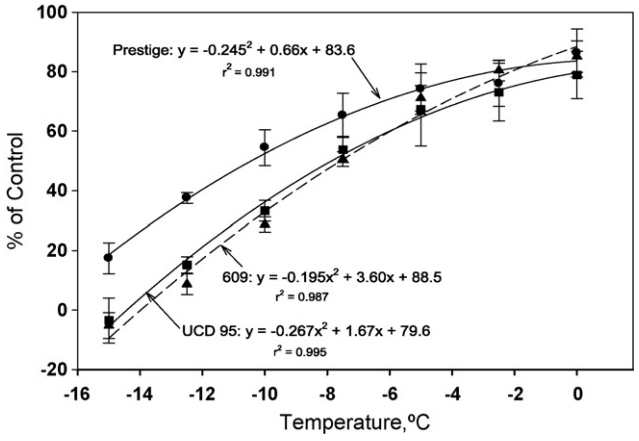

Fig. 1. Relative metabolic heat production rates of stolon segments of 'Prestige', 609 and UCD 95 buffalograss genotypes measured at 20 °C following low-temperature treatments of 0, −2.5, −5, −7.5, −10, −12.5 and −15 for 1 h each.

3.2. Scanning microcalorimetry

The results from the scans from 2 to −25 °C using leaf segments are shown in Fig. 2. Peaks indicate when leaf segments froze, giving off heat in the process. When more than one segment froze at any given temperature, the number of segments freezing was estimated by the height of the peak. By using this approach all 20 leaf segments for each genotype could be accounted for in Fig. 2. The mean freezing temperatures of leaf segments from each of the three genotypes were calculated by summing the temperatures at which each leaf segment froze (indicated by its peak) and dividing by the number of segments. By following this procedure we found that the mean freezing temperature for leaf segments from genotypes 'Prestige', 609 and UCD 95 were −18.7, −16.3 and −15.0 °C, respectively (Fig. 2). The majority of UCD 95 leaf segments froze around −14.5 °C; however, a few froze at lower temperatures with two freezing at approximately −18 °C. Leaf segments from genotypes 609 and 'Prestige' froze over a wider range of temperatures ranging from about −14 °C down to approximately −22 °C.

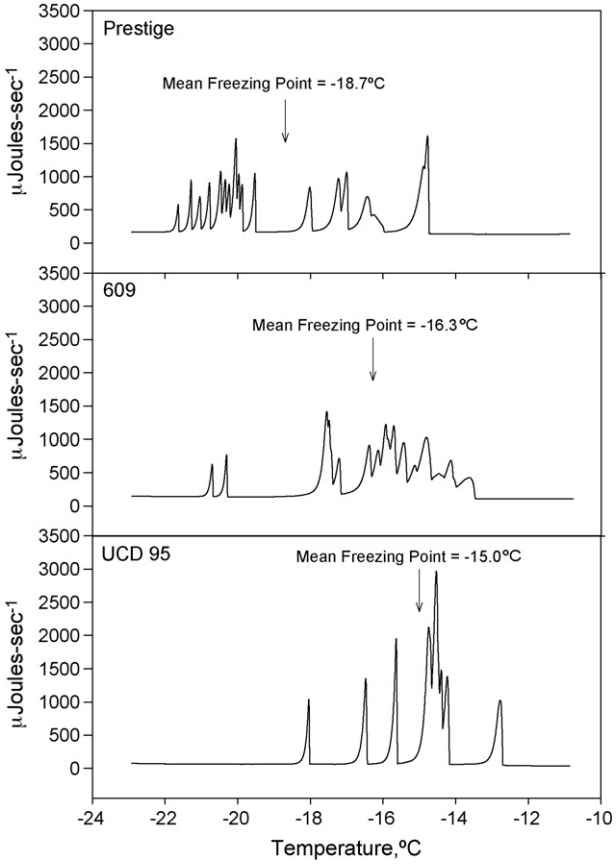

Fig. 2. Scanning microcalorimetry of leaf segments of 'Prestige', 609 and UCD 95 showing temperatures at which freezing occurred. $N = 20$ for each genotype.

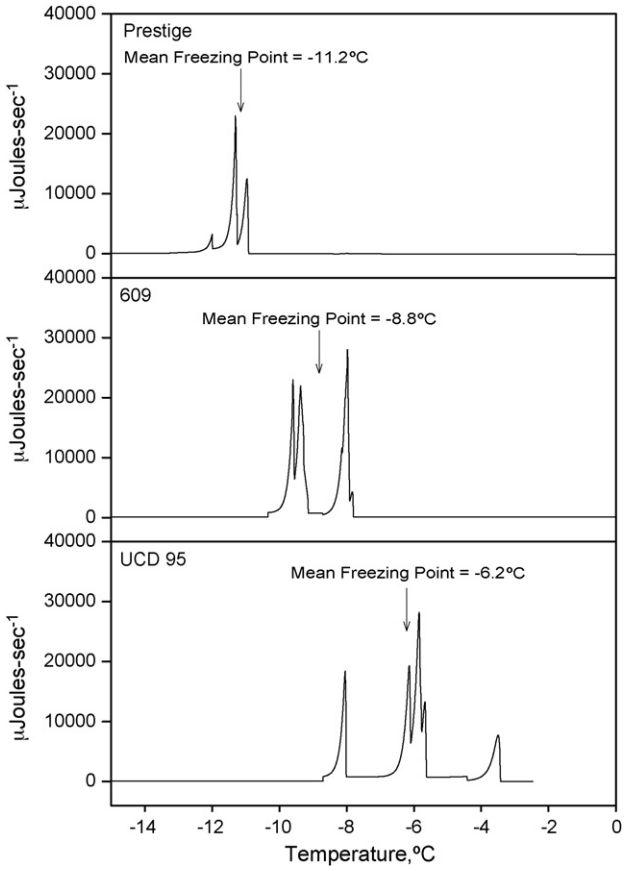

Fig. 3. Scanning microcalorimetry of root segments of 'Prestige', 609 and UCD 95 showing temperatures at which freezing occurred. $N = 15$, 24 and 24 for genotypes 'Prestige', 609 and UCD 95, respectively.

The mean freezing temperatures for root segments of genotypes 'Prestige', 609 and UCD 95 were −11.2, −8.8 and −6.2 °C, respectively (Fig. 3).

Almost all UCD 95 root segments froze before those of either 609 or 'Prestige' in temperatures between approximately −3 and −8 °C. There was a little overlap in the freezing temperatures of 609 and UCD 95 at approximately −8 °C, but no overlap in freezing temperatures between 609 and 'Prestige'. The temperature ranges at which 609 and 'Prestige' root segments froze were very narrow with those from 609 freezing at −9 °C and those from 'Prestige' freezing at approximately −11 °C. In general, the mean freezing points for root segments were 7.5–8.8 °C higher than those for leaf segments for each genotype.

3.3. Genotype freeze testing—determination of LT_{50}

A comparison of the calorimetric data with actual freezing responses showed similar results. Fig. 4 shows the LT_{50} for 'Prestige', 609 and UCD 95 genotypes. At every sampling date lower temperatures were required to reach the LT_{50} for 'Prestige' than for either 609 or UCD 95. These latter two genotypes differed in LT_{50} only when sampled in January.

4. Discussion

With isothermal microcalorimetric measurements, we found that buffalograss genotype 'Prestige' was more tolerant of low-temperature exposures and began to differ from genotypes 609 and UCD 95 at temperatures between −7.5 and −10 °C. This result coincided with actual measurements of LT_{50} (Fig. 4) that showed that lower temperatures (approximately 5–7 °C lower) were required to reach the LT_{50} for 'Prestige' than for 609 or UCD 95. Field observations (data not presented) showed that 'Prestige' suffered substantially less winter injury in the field in Colorado than 609 and UCD 95. The results from this study suggest that the ability of 'Prestige' to maintain a more stable metabolic activity in response to cold temperature contributed to its greater low-temperature tolerance than that of 609 and UCD 95.

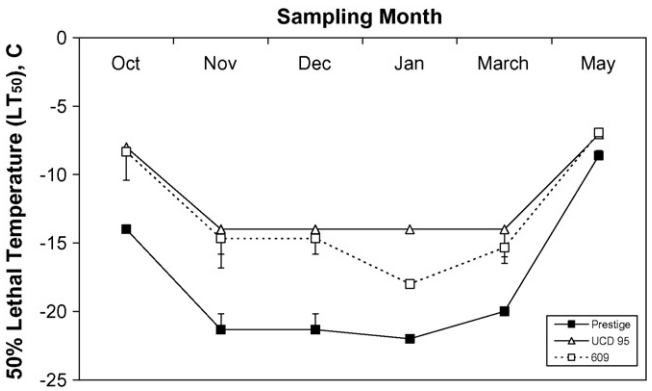

Fig. 4. Response of 'Prestige', 609 and UCD 95 buffalograss genotypes to freezing temperatures between −5 and −25 °C. Temperature values for each sampling are the temperatures at which 50% of the stolons were killed (LT_{50}).

Scanning microcalorimetry indicated that leaf and root segments of 'Prestige' froze at lower temperatures than 609 and UCD-95 although considerable variations in leaf freezing point existed. Previously, Ball et al. [1] found that genotype 'Prestige' contained significantly higher levels of glucose, fructose, and raffinose than genotype 609. These soluble carbohydrates likely contributed to the reduced freezing points of roots and leaves. Freezing point depression provides an effective, low-temperature protective mechanism by avoiding many of the damaging effects of ice formation.

Overall, UCD 95 was the least tolerant of low-temperature exposures and its tissues froze at the highest temperature of the three genotypes tested in this study. 'Prestige' was the most tolerant of low-temperature exposures and its tissues froze at the lowest temperature; genotype 609 was intermediate. These results support the findings of Wu and Harivandi [22] where they focused on two "Mexico" selections (one of which became UCD 95) and the recent work of Qian et al. [16] and Ball et al. [1] where they found that 'Prestige' survived 4–5 °C colder temperatures than 609. Therefore, the results obtained in the present study were predictive of the actual low-temperature tolerance of these three genotypes.

In the present study actively growing stolons, leaves and roots were tested for their relative freezing responses to low temperatures. Acclimatization techniques were not employed so as to not confuse the inherent response of the genotypes and tissues in question to low temperatures. It is possible that the three genotypes and tissues used in this study would have responded differently to sub-zero temperatures after having been acclimatized. Instead, our results show the relative inherent differences among the three genotypes.

5. Conclusion

This microcalorimetric approach provided an ability to rapidly rank three buffalograss genotypes with regard to their low-temperature tolerance. We based this ranking on: (1) their ability to withstand low temperatures and (2) the temperatures at which

their leaf and root tissues froze. Our ability to associate microcalorimetric results with actual low-temperature survivability parallels work with *Pyrus* [18] and *Malus* [17]. The calorimetric techniques utilized here for estimating and predicting low-temperature effects on plant metabolism and tissue freezing points hold great promise for those interested in determining the relative tolerance of whole plants and plant tissues to low temperatures.

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