

Screening Cold Tolerance in St. Augustinegrass (*Stenotaphrum secundatum* (Walt.) Kuntze) for USDA Hardiness Zone 7

Research Article

Volume 4 Issue 1- 2023

Author Details

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Article History

Received: April 13, 2023 Accepted: April 18, 2023 Published: April 19, 2023

Abstract

Among warm-season turfgrasses, St. Augustinegrass [*Stenotaphrum secundatum* (Walt.) Kuntze] generally has poor cold tolerance yet excellent shade tolerance. As mostly hot summers follow cold winters in USDA Hardiness Zone 7, severely damaging tall fescue [*Festuca arundinacea* Schreb.] and centipedegrass [*Eremochloa ophiuroides* (Munro) Hack.], a St. Augustinegrass cultivar cold tolerant enough to be grown for shady lawns would greatly benefit both home owners and sod growers in this and potentially other regions. Eight St. Augustinegrass samples were selected from an initial collection of 30, including industry standards 'Raleigh' and 'Palmetto', for further testing from an established germplasm collection of material collected from lawns grown in USDA Hardiness Zone 7. Percent regrowth was calculated 4 weeks after samples underwent a 2 week cold acclimation process, followed by freezing at 0°C, -4°C, and -6°C separately for 3 h. The germplasm samples designated at 'A', 'G', and 'H' had greater clipping regrowth percentages compared to the industry cold tolerant standard, 'Raleigh' at either -4°C and -6°C indicating possible increased cold tolerance for several selections compared to 'Raleigh'. An increased cold tolerant St. Augustinegrass would provide a wider selection of desirable turfgrasses in the transition zone growing region of the world, especially shady sites.

Abbreviation: USDA: United States Department of Agriculture

Introduction

St. Augustinegrass [*Stenotaphrum secundatum* (Walt.) Kuntze] is one of the most popular turfgrass species used for home lawns in tropical and subtropical regions of the world [1]. It is believed to be native to open-to-lightly shaded, high rainfall, and humid regions of coastal South and Central America including the West Indies [2]. This species is adaptable to many soil conditions, but does best on moist, well-drained sandy soils. Irrigation is necessary during periods of dry weather as its drought tolerance is only considered fair [3]. On occasion, USDA Hardiness Zone 7 and other transition regions of the world experience years of drought and above average heat during summer months, followed by below average cold temperatures during winter months. Often, these conditions create a turf void in shady locations throughout the landscape. Such areas suffer as temperatures become too hot for tall fescue [*Festuca arundinacea* Schreb.], a C3 plant, survival, yet too cold for centipedegrass [*Eremochloa ophiuroides* (Munro) Hack.], a C4 plant, survival. For Clemson, SC, average

summer high and low temperatures are 32°C and 20°C, with a record high of 41°C. Average winter high and low temperatures are 11°C and -1°C, with a record low of -20°C [4].

Warm-season grasses exhibit optimum growth between 27°C and 38°C as cool-season grasses exhibit optimum growth when temperatures range between 15°C and 25°C [2]. As summer nights fail to cool below 18°C, tall fescue struggles to recover from daytime heat. Permanent turfgrass injury to warm-season turfgrass, such as centipedegrass, often occurs if ambient temperatures drop rapidly below -5°C and gradually below -12.2°C [2]. This weather pattern, combined with a growing population in the upstate, opens a niche demand for a warm season (C4) grass cold tolerate enough to survive below average temperatures in shaded lawns. Shade and cold tolerance are a must for new turfgrass species to fill demands of this niche market. Winter-kill is often a problem in St. Augustinegrass sod production [5], as St. Augustinegrass is the least freezing-tolerant of the warm-season turfgrasses [6]. St. Augustinegrass traditionally has been USDA hardiness zones 8, 9, and 10. However, severe freezing injury may occur during periodic winters in zones 8 and 9. Improvement of cold tolerance in



St. Augustinegrass would increase the area of adaptation and potential use of this important turfgrass species [5].

The survival of plants rely on the ability of its meristematic tissue to survive freeze injury. Freezing temperatures resulting in ice formation within plant cells can cause multiple types of tissue damage and death of the entire plant under severe conditions [7]. During a period of low but non-freezing temperatures in a process called cold-acclimation [7-9], plants can increase their ability to withstand freezing temperatures. In nature, cold-acclimation is initiated by decreasing temperatures in late autumn or early winter.

Selecting plants with increased tolerance to winter freezing is an important aspect of plant improvement. However, fluctuating winter temperatures make it necessary for experiments to be conducted in multiple locations and years [10]. Such tests are costly and time-consuming. Therefore, cold tolerance evaluations have been conducted in field trials, cold simulation chambers [6] and by excised stolon regrowth tests [11,12]. Germplasm collection is an effective approach for cultivar development [13]. For example, tall fescue (*Festuca arundinacea* Schreb. cv. 'Kentucky 31'), one of the most popular tall fescue cultivars, and 'Raleigh', currently the most cold tolerant St. Augustinegrass cultivar, were both selected from plants collected from the field [11].

The purpose of this study was to evaluate cold tolerance of a St. Augustinegrass germplasm collection from upstate South Carolina, for potential sod production and subsequent lawn use in the transition zone. Comprehensive evaluations of these plant collections could open new opportunities for sod growers to provide homeowners with a highly shade tolerant warm season turfgrass capable of surviving unusually cold winters in USDA Hardiness Zone 7.

Materials and Methods

In 2002, a germplasm collection was established with samples from 30 St. Augustinegrass lawns grown in USDA Hardiness Zone 7. These samples, along with commercial standards, 'Raleigh' and 'Palmetto', were established by plugs under natural low light (~50% full sunlight) conditions in Clemson, South Carolina (34°40'14" N, 82°50'15" E). In June of 2012, plugs from the top eight performing grasses were collected and transplanted into 24 plastic trays (53x38x8cm), filled with river sand, using four, 5cm, plugs per tray. Trays were transported to Clemson University's Greenhouse Facility and grown for 12 months at 25+10°C. Sprigs of each grass sample were transplanted into 10 cm diameter pots filled with a potting soil medium (Fafard 3B mix, Concord Fafard Inc., Agawam, MA, USA) using six sprigs with 3 internodes and 8-12cm in length, per pot. Pots were placed in a growth chamber and established for 6 weeks at 32°C with a 16 h photoperiod (500 $\mu\text{mol m}^{-2} \text{s}^{-1}$). Pots were fertilized at 25kg N/ha on three week intervals using a 1-1-1 complete fertilizer, watered every other day to field capacity, and mowed at 5.5cm, twice weekly. Pots were selected for preliminary testing to identify the target freezing temperature by exposing plants to -2°C, -4°C, -6°C, and -8°C for 3h following the 'two-step acclimation' protocol to simulate the natural acclimation in late fall or winter [12,13]. Greater than 95% of plants provided regrowth at -2°C, while <10% of plants provided regrowth at -8°C. Therefore, both -4°C and -6°C were selected as optimum temperatures for testing as 60% of plants provided regrowth at -4°C and 20% of plants provided regrowth at -6°C.

Remaining pots were relocated into three separate growth chambers and simultaneously subjected to a cold acclimation period. Growing conditions were reduced to 13°C with a photoperiod of 12h for one week. This period was followed by a temperature reduction to 3°C and photoperiod reduction of 10h for another week. The control growth chamber was maintained at 3°C. Growth chambers were then lowered at 1°C h⁻¹ to target temperature (-4°C and -6°C) and maintained for 3h. Temperatures were then raised back to 3°C at 2°C h⁻¹. After three hours at 3°C, pots were moved into the greenhouse. Plants re-

covered for four weeks during a regrowth period under natural light, at 25°C+5°C. Plants were then mown at 5.5cm, clippings collected, dried for 48 hours at 60°C, and weighed (g). Regrowth weight was calculated as a percentage to control (cold acclimation only) plant's growth weights. Pots were labeled 'A' through 'H' with 'Raleigh' designated as 'B', and 'Palmetto' designated as 'D'. All samples were grown in 10 cm diameter pots, allowed to establish under optimum growing conditions in the growth chamber, then underwent the two-step acclimation process before freezing. Pots were rotated within the three growth chambers every week to avoid localized environmental effects. Mean clipping weights were calculated at each temperature (0°C, -4°C, -6°C), then divided by mean clipping weights at 0°C to calculate a percent regrowth. Percent regrowth's were then compared to 'Raleigh's percent regrowth to determine improved cold tolerance versus the industry standard in this region.

Experimental design was a completely randomized design including three temperature regime treatments: cold acclimation, cold acclimation followed by a freezing period of -4°C, and cold acclimation followed by a freezing period of -6°C and six replications of each grass sample were used. Calculations were performed using the NLMIXED Procedure in SAS version 9.3 to compare regrowth percentages (SAS Institute, Inc., Cary, NC). Regrowth percentages of experimental grasses were compared to industry standard 'Raleigh' to determine statistically similar or significant differences ($P < 0.05$). Study one was conducted from 2 December 2013 thru 17 January 2014, with study two from 31 January 2014 thru 17 March 2014.

Results and Discussion

Significant interactions occurred between the two studies; therefore, data are presented separately. In study one, at -4°C, 'Raleigh' regrew 90% compared to its clipping weight at 0°C, while six grass samples had statistically similar regrowth compared to 'Raleigh' including: 'C', 'Palmetto', 'E', 'F', 'G' and 'H' (Figure 1). Sample 'A' had a significant (67%) increase in regrowth compared to 'Raleigh', at -4°C. 'A' regrew 156% compared to its clipping weight at 0°C. In study one, at -6°C, 'Raleigh' regrew 38% compared to its clipping weight at 0°C and all grass samples showed statistically similar regrowth compared to 'Raleigh' (Figure 2). Although statistically similar, sample 'A' regrew 43% compared to its clipping weight at 0°C, which was the only sample with numerically greater regrowth (5%), compared to 'Raleigh'. In study two, at -4°C, 'Raleigh' regrew 70% compared to its clipping weight at 0°C and all grass samples showed statistically similar regrowth compared to 'Raleigh' (Figure 3). Although statistically non-significant ($P < 0.05$), grass 'H' did have numerically greater regrowth compared to 'Raleigh' with a p-value of 0.069. This is worth noting due to its significant regrowth compared to 'Raleigh' at -6°C, during study two (Figure 4). In contrast to study one, at -4°C, Sample 'A' had similar regrowth compared to 'Raleigh'.

In study two, at -6°C, 'Raleigh' again regrew 38% compared to its clipping weight at 0°C (Figure 4). Five grass samples had statistically similar regrowth compared to 'Raleigh' including: 'A', 'C', 'Palmetto', 'E', and 'F'. Two samples, 'G' and 'H', had significant increased regrowth compared to 'Raleigh' of 56% and 87%, respectively. Although 'Palmetto' regrew more than 'G' numerically (59%), its large standard error reveals statistically similar regrowth compared to 'Raleigh' unlike sample 'G', with a smaller standard error. North Carolina is the northern edge of St. Augustinegrass distribution range [13]. 'Raleigh', a release from North Carolina State University, is considered the most cold-tolerant cultivar currently available [1,5,12]. However, the use of 'Raleigh' is limited to areas that rarely experience temperatures lower than -5°C [13]. Fry et al. [14] reported lethal temperatures for 'Flor-atam' to vary monthly from -6.1°C to -5.3°C between December and March in Louisiana. In contrast, Murdoch, et al. [15] reported 'Flor-atam' nodes from actively growing turf were killed following exposure to -4°C.



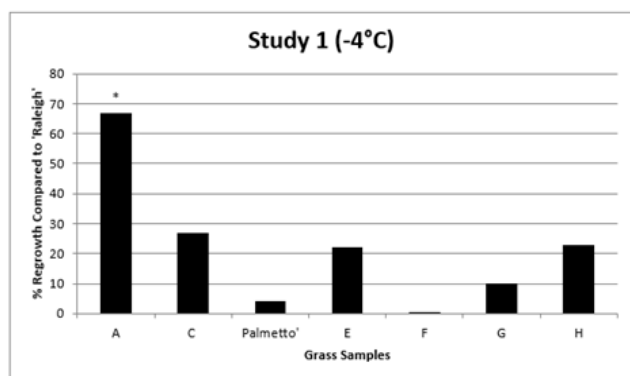


Figure 1: Study 1 regrowth percentage of seven germplasm samples frozen at -4°C for 3h after two-step cold acclimation under controlled growth chamber conditions. Columns represent mean percent regrowth subtracted from mean percent regrowth of 'Raleigh' of six replicates. Grass samples containing (*) were significantly different from 'Raleigh' according to the NLMIXED Procedure in SAS ($P=0.05$).

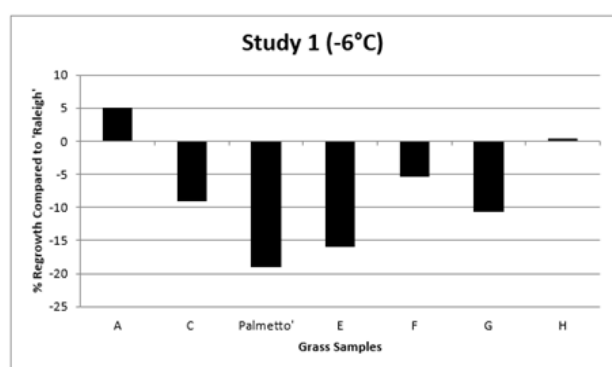


Figure 2: Study 1 regrowth percentage of seven germplasm samples frozen at -6°C for 3h after two-step cold acclimation under controlled growth chamber conditions. Columns represent mean percent regrowth subtracted from mean percent regrowth of 'Raleigh' of six replicates. Grass samples containing (*) were significantly different from 'Raleigh' according to the NLMIXED Procedure in SAS ($P=0.05$).

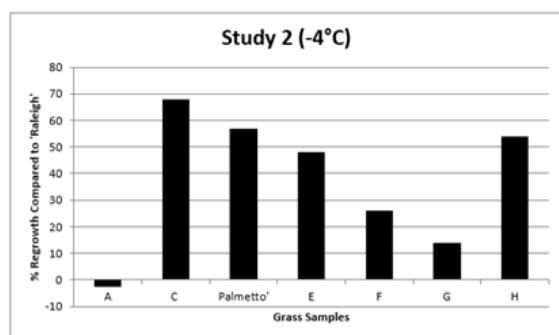


Figure 3: Study 2 regrowth percentage of seven germplasm samples frozen at -4°C for 3h after two-step cold acclimation under controlled growth chamber conditions. Columns represent mean percent regrowth subtracted from mean percent regrowth of 'Raleigh' of six replicates. Grass samples containing (*) were significantly different from 'Raleigh' according to the NLMIXED Procedure in SAS ($P=0.05$).

Our effort was to determine if plant material collected from USDA Hardiness Zone 7 provided improved cold tolerance. In one of two studies, three samples, 'A', 'G', 'H' provided statistically greater regrowth rates compared to current industry standard 'Raleigh' with similar regrowth rates in another study. Various methods have been developed to predict, or correlate to, freezing tolerance in St. Augustinegrass, which include electrolyte leakage technique [16], meristematic regeneration within stolon nodes, and differential thermal

analysis [12,17]. In our experiments, we measured clipping weights and divided by clipping weights at 0°C to calculate a percent regrowth at 4 weeks after freezing. Cold-acclimation has been shown to be a crucial prerequisite for plants to survive freezing temperatures in nature as well as in laboratory tests [13]. However natural acclimation is impossible to duplicate because acclimating conditions vary from year to year. Li, et al. [13] suggested the two-step acclimation protocol closely assimilates the natural acclimation period. Incorporating this cold acclimation protocol with plugs grow in 10 cm pots, opposed to individual internodes, provided the most comparable methods to natural freezing by using a controlled environment.

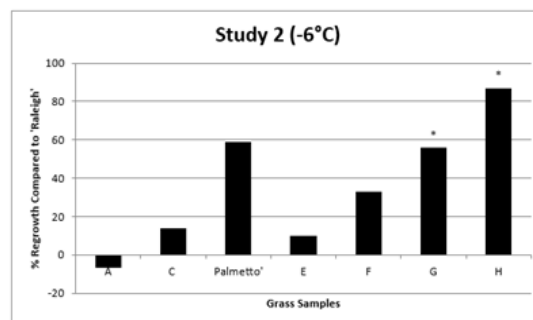


Figure 4: Study 1 regrowth percentage of seven germplasm samples frozen at -6°C for 3h after two-step cold acclimation under controlled growth chamber conditions. Columns represent mean percent regrowth subtracted from mean percent regrowth of 'Raleigh' of six replicates. Grass samples containing (*) were significantly different from 'Raleigh' according to the NLMIXED Procedure in SAS ($P=0.05$).

Conclusions

These experimental grass samples appear to have similar or improved cold tolerance, especially grasses 'A', 'G', and 'H', compared to the industry standard 'Raleigh'. Field studies are needed to validate greenhouse growth chamber studies to help further evaluate cold tolerance of experimental and commercial lines. However, these lines appear to potentially expand the usable area in terms of cold tolerance of St. Augustinegrass throughout the world.

Acknowledgements

Technical Contribution No. 7169 of the Clemson University Experiment Station. This material is based upon work supported by the NIFA/USDA, under project number SC-1700607. Dr. Patrick Gerard provided experimental design and statistical analysis assistance.

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