HORTSCIENCE 29(4):316–318. 1994.

Evaluation of an Electrolyte Leakage Technique to Predict St. Augustinegrass Freezing Tolerance

F.P. Maier and N.S. Lang
Department of Horticulture, Louisiana Agricultural Experiment Station, Louisiana State University Agricultural Center, Baton Rouge, LA 70803

J.D. Fry
Department of Horticulture, Forestry, and Recreation Resources, Kansas State University, Manhattan, KS 66506

Additional index words. cold tolerance, turfgrass, *Stenotaphrum secundatum*

Abstract. **Stolons of 'Raleigh', 'Floratam', and FX-332 St. Augustinegrass *[Stenotaphrum secundatum* (Walt.) Kuntze] were sampled from the field between October and March in two consecutive years to evaluate accuracy of an electrolyte leakage (EL) method for predicting freezing tolerance. Lethal temperatures of stolons estimated using EL were compared to those obtained by regrowth tests in the greenhouse. Mean lethal low temperatures for regrowth and EL methods over 12 sampling dates were 'Floratam', –4.5C (regrowth) vs. –4.4C (EL); FX-332, –4.2C (regrowth) vs. –4.9C (EL); and 'Raleigh', –6.0C (regrowth) vs. –5.4C (EL). A positive correlation (r = 0.81) was observed between EL-predicted and regrowth lethal temperatures for 'Raleigh', which exhibited some acclimation during the first sampling year. The EL technique consistently predicted a lower lethal temperature for 'Raleigh' than for 'Floratam', which corroborates field observations concerning freezing tolerance of these two cultivars.**

St. Augustinegrass is commonly used as a turfgrass in landscapes in the southern United States. Because of poor freezing tolerance, its use is limited to areas that rarely experience temperatures less than –5C. Researchers have evaluated turfgrass freezing tolerance by observing recovery in field trials (Wilson et al., 1977); exposing grasses to freezing temperatures in a controlled environment and evaluating regrowth in the greenhouse (Fry et al., 1993); using triphenyl tetrazolium chloride as an indicator of survival (Ahring and Irving, 1969); and by measuring electrolyte leakage (EL), which is an indicator of cell injury (Ahring and Irving, 1969).

Although field evaluations provide reliable information on turfgrass freezing tolerance, they may be undesirable because plot maintenance is costly, and low temperatures needed to differentiate freezing tolerance may not occur every year.

Evaluation of regrowth after exposure to a predetermined temperature regime has been used to determine lethal low temperatures in bermudagrass *[Cynodon dactylon* (L.) Pers.] (Adams and Twersky, 1960; Anderson et al., 1988; Davis and Gilbert, 1970; Dunn and Nelson, 1974; Gilbert and Davis, 1971; Ibitayo et al., 1981; Reeves et al., 1970); zoysiagrass *(Zoysia* spp.) (Rogers et al., 1975, 1977); and St. Augustinegrass (Reeves and McBee, 1972). However, regrowth evaluations take 4 to 6 weeks to complete and are only accurate within a range of selected treatment temperatures.

Dexter et al. (1932) developed an EL method to predict freezing tolerance of alfalfa *(Medicago sativa* L.) roots. Cells killed by freezing temperatures leak electrolytes and other cell contents as a result of membrane disruption. The extent of EL is used to estimate freezing injury. This procedure is considerably faster than field evaluations and regrowth tests. EL has provided a good estimate of lethal low temperatures infield-grown bermudagrass (Anderson et al., 1988; Ibitayo et al., 1981; Sowers, 1986) and underestimated centipedegrass *[Eremochloa ophiuroides* (Munro) Hack] freezing tolerance by ≈2C (Fry et al., 1993).

An EL method similar to one described by Anderson et al. (1988) was used to predict lethal low temperatures in 'Floratam' St. Augustinegrass (Fry et al., 1991; Murdoch et al., 1990) with good results. However, the effectiveness of EL in determining differences in freezing tolerance among St. Augustinegrass cultivars is unknown. The objective of this study was to evaluate an EL technique for accuracy in predicting intraspecific differences in freezing tolerance of 'Raleigh', 'Floratam', and FX-332 St. Augustinegrass. 'Raleigh' is marketed as a freezing-tolerant cultivar, while 'Floratam' is considered to have poor freezing tolerance. FX-332 is a fast-growing, experimental cultivar from the Univ. of Florida that was established in field plots at Baton Rouge, La., in 1989. It survived a Christmas-day freeze during which the air temperature dropped to -13C and most other cultivars died.

Received for publication 21 June 1993. Accepted for publication 3 Nov. 1993. Paper no. 93-28-7199 of the Journal Series, Louisiana Agricultural Experiment Station. Kansas Agricultural Experiment Station contribution no. 93-501 -J. 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

Materials and Methods

Stolons were collected monthly from the field between Oct. and Mar. 1990-91 and 1991–92. Turfgrass sampled was part of the National Turfgrass Evaluation Program St. Augustinegrass variety trial established in 1990 at the Burden Research Plantation in Baton Rouge. The turf was established on an Olivier silt loam (fine-silty, mixed, thermic aquic, Fragiudalf), fertilized with 49 kg N/ha in April and July of each year, maintained at a 5-cm height, and irrigated as needed to prevent drought stress. Stolons were sampled simultaneously for freezing tolerance evaluation by regrowth and EL methods.

To determine freezing tolerance by regrowth, stolons with roots and leaves intact were separated into 15-cm-long segments, each with four nodes, and were washed with tap water. Ten stolons were placed in a plastic resealable -bag. Each bag was considered a replication with three replications included per treatment temperature and cultivar. Bags were randomly placed in a low-temperature incubator (C 1213, Curtin Matheson Scientific, Houston) with thermocouples randomly inserted in five bags to monitor temperature. The incubator remained at 1C overnight. The following day, chamber temperature was reduced 2C/h. Three replicate bags of each cultivar were removed following 1-h exposure to –2, –4, -6, and –8C. A 1C treatment was used as a control. Following temperature treatments, stolons were placed in a 1C incubator to thaw slowly overnight. Stolons were planted in a 1 peat : 1 perlite (v/v) medium, placed in a greenhouse (air mean maximum/minimum, 27C/18C), and watered daily to maintain adequate soil moisture. Stolons were harvested after 4 to 6 weeks, and nodes where shoot regrowth occurred were counted. Nodes that were green and turgid but exhibited no shoot regrowth were included in regrowth counts. The lethal temperature range was defined as the two temperatures between which node regrowth dropped to <50% (Lt_{50}).

o determine freezing tolerance by EL, 10-cm-long stolons of 'Floratam', 'Raleigh', and FX-332 were washed with tap water, roots and leaves were removed, and each stolon was trimmed into segments containing three nodes. Stolons were rewashed with distilled, deionized water; wrapped in a moist paper towel; and placed in 12 × 150-mm culture tubes that were kept in an incubator at 1C overnight. The following day, tubes were arranged randomly in a 1 ethylene glycol : 1 distilled water (v/v) bath (model RM20; Brinkman Instruments, Westbury, N.Y.) preset to 1C. Thermocouples were randomly inserted in tubes to monitor temperature. Small ice chips were placed in each tube to encourage ice nucleation, thereby ensuring measurement of freezing tolerance and not avoidance caused by possible supercooling of tissue liquid. Treatment temperatures and rate of temperature drop were as described previously. Ten replicate tubes of

each cultivar were removed after 1-h exposure to each temperature and placed in a 1C incubator to thaw overnight.

Each stolon segment was removed from the tube, and two nodes were excised. Excised nodes were placed in tubes, each containing 10 ml distilled, deionized water. Freezing-induced electrical conductivity (EC) of node leachate was measured on the following day using a solution analyzer (Cole-Parmer Instrument Co., Chicago). To determine potential EC, tubes were held at 80C in a water bath for 1 h. After samples were held at 27C overnight, EC of the leachate was measured. Percent EL was determined for each cultivar at each treatment temperature: EL (%) = freezing-induced EC/potential EC × 100.

Lethal temperatures were determined using the Gauss–Newton method of nonlinear regression available in the PROC NLIN procedure (SAS Institute, 1985). Data were fitted with a sigmoidal response curve using a model developed for plant heat-stress studies (Ingram, 1985). The inflection point of the sigmoidal response curve estimates the lethal temperature.

Correlation analysis was run between lethal temperatures generated from the EL and regrowth methods for each cultivar. The midpoint of the regrowth lethal temperature range was used in correlation analysis and determination of an average lethal low temperature.

Results and Discussion

Lethal temperatures determined by EL ranged from –4.0C in Oct. 1990 for 'Floratam' to –6.8C in Nov. 1991 for 'Raleigh' (Fig. 1). Only 'Raleigh' exhibited EL-predicted lethal temperatures less than –6.0C. With the exception of 'Raleigh' in 1990–91, EL-predicted lethal temperatures for each cultivar did not vary by >1C among winter months. The EL-predicted lethal temperature was within the Lt_{50} range estimated by the regrowth method on 10 of 12 sampling dates during the two winters for 'Floratam', 7 of 12 dates for FX–332, and 12 of 12 dates for 'Raleigh' (Fig. 1).

A significant positive correlation ($r = 0.81$) existed between EL-predicted and regrowth lethal temperatures for 'Raleigh'. 'Raleigh' was the only cultivar that exhibited acclimation, as indicated by an EL-predicted lethal temperature of –3.8C in Oct. 1990 compared to –6.0C in Jan. 1991 (Fig. 1). Little change in freezing tolerance was observed for 'Floratam' and FX-332 over sampling dates; hence, poor correlation coefficients resulted when the two methods were compared.

EL-predicted lethal temperatures were lower than the regrowth Lt_{50} range on all dates that values did not agree (Fig. 1). The majority of overestimates of freezing tolerance by EL was observed in FX-332. In centipedegrass, electrolytes were leaked gradually between –4 and –10C, which resulted in an inflection point that slightly underestimated the lethal temperature (Fry et al., 1993). A similar, gradual loss of electrolytes over a range of freezing temperatures seems to occur in FX-332 St. Augustinegrass.

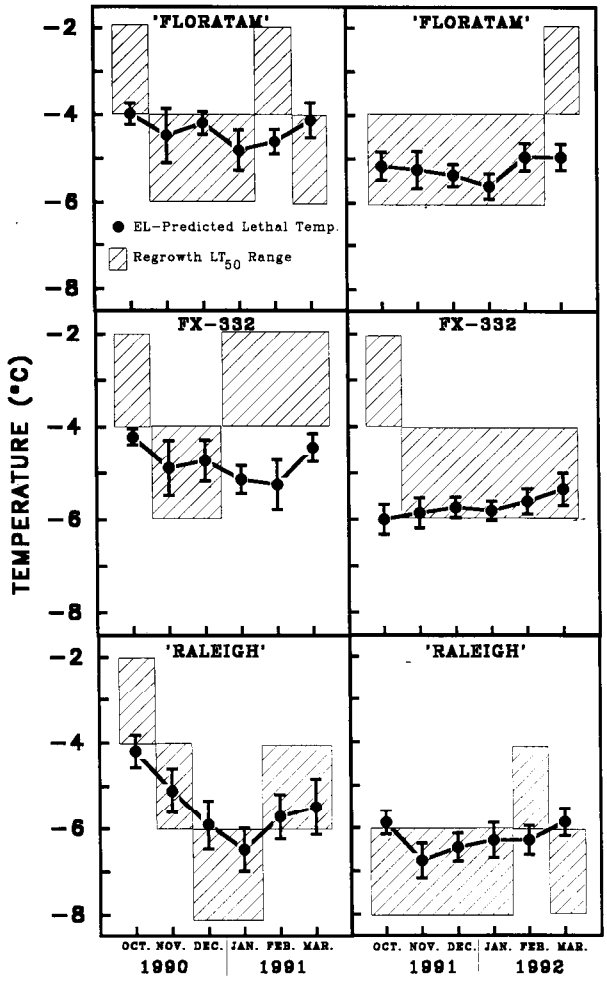

Fig. 1. Comparison of regrowth and EL methods for determining freezing tolerance of 'Floratam', FX-332, and 'Raleigh' St. Augustinegrass in 1990–91. Points represent the lethal temperature predicted by EL; intersecting bars indicate the 95% confidence interval. Shaded areas covering 2C intervals represent the Lt_{50} range determined by regrowth.

The regrowth Lt_{50} range for 'Raleigh' was lower than that of 'Floratam' and FX-332 on 8 of 12 dates sampled. Our results confirmed that 'Raleigh' is more freezing tolerant than 'Floratam'. This maybe due, in part, to lower stolon water content in 'Raleigh' compared to 'Floratam' during winter months (Maier et al., 1993). Lethal temperatures estimated by EL for 'Floratam' in this study were similar to those reportedly Fry et al. (1993) and Murdoch et al. (1990). FX-332 was no more freezing tolerant than 'Floratam'. Survival of FX-332 in field plots in Dec. 1989 may have been due to prolific stolon production that provided insulation from low temperatures. Further, the large number of nodes produced increased the likelihood that a few would survive.

Wilson et al. (1977) reported following field evaluation that 'Raleigh' is more freezing tolerant than 'Floratam'. EL, used in the present study to support these results, is a useful technique for rapid determination of intraspecific differences in St. Augustinegrass freezing tolerance.

Literature Cited

Adams, W.E. and M. Twersky. 1960. Effect of soil fertility on winter killing of coastal bermudagrass, Agron. J. 52:325–326.

Ahring, R.M. and R.M. Irving. 1969, A laboratory method of determining cold hardiness in bermudagrass, *Cynodon dactylon* (L.) Pers. Crop Sci. 9:615–618.

Anderson, J.A., M.P. Kenna, and C.M. Taliaferro, 1988. Cold hardiness of 'Midiron' and 'Tlfgreen' bermudagrass. HortScience 23:748-750.

Davis, D.L. and W.B. Gilbert. 1970. Winter hardiness and changes in soluble protein fractions of bermudagrass. Crop Sci. 10:7-9.

Dexter, S.T., W.E. Tottingham, and L.F. Graber. 1932. Investigations of the hardiness of plants by measurement of electrical conductivity. Plant Physiol. 7:63-78.

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 'Floratam' St. Augustinegrass. HortScience 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.

Gilbert, W.B. and D.L. Davis. 1971. Influence of fertility ratios on winter hardiness of bermudagrass. Agron. J. 63:591–593.

Ibitayo, O.O., J.D. Butler, and M.J. Burke. 1981. Cold hardiness of bermudagrass and *Paspalum vaginatum* Sw, HortScience 16:683–684.

Ingram, D.L. 1985. Modeling high temperature and

exposure time interactions on *Pittosporum tobira* root cell membrane thermostability. J. Amer. Soc. Hort. Sci. 110:470–473.

Maier, F.P., N.S. Lang, and J.D. Fry. 1993. Freezing tolerance of three St. Augustinegrass cultivars as affected by stolon carbohydrate and water content. J. Amer. Soc. Hort. Sci. (In press,)

Murdoch, C.L., A.E. Dudeck, and C.L. Guy. 1990. Development of a technique for screening St. Augustinegrass for cold tolerance. Turfgrass Res. Fla. p. 52–63.

Reeves, S.A. and G.G. McBee. 1972. Nutritional influences on cold hardiness of St. Augustinegrass *(Stenotaphrum secundatum).* Agron. J. 64:447–450.

Reeves, S.A., G.G. McBee, and M.E. Bloodworth. 1970. Effect of N, P, and K tissue levels and late fall fertilization on the cold hardiness of 'Tifgreen' bermudagrass *(Cynodon dactylon* × *C. transvalensis).* Agron. J. 62:659–662.

Rogers, R.A., J.H. Dunn, and C.J. Nelson. 1975. Cold hardening and carbohydrate composition of Meyer zoysia. Agron. J. 67:836–838.

Rogers, R.A., J.H. Dunn, and C.J. Nelson. 1977. Photosynthesis and cold hardening in zoysia and bermudagrass. Crop Sci. 17:727–732.

SAS Institute. 1985. The NLIN procedure, p.575–614. In: SAS user's guide: Statistics. Version 5 edition. SAS Inst., Cary, N.C.

Sowers, R.S. 1986. Electrolytic evaluation of bermudagrass cold hardiness and microenvironmental modification for seasonal establishment of bermudagrass. MS thesis, Dept. of Agronomy; Univ. of Maryland, College Park.

Wilson, C.A., J.A. Reinert, and A.E. Dudeck. 1977. Winter survival of St. Augustinegrass in north Mississippi, Southern Turfgrass Assn. Bu1. 12:20.