The "rate" treatment was identical to the "shock" treatment with the following exceptions. All leaf samples (60) for the former were placed in the 25°C water bath at once. The bath temperature was increased at a rate of 5° per hr. This rate is similar to maximum values observed in the field in Florida citrus growing regions. Six samples (three per species) were removed as the tissue reached one of each of the 10 desired treatment temperatures up to 65°. Thereafter, the leaves were cut into strips and subjected to procedures described for the "shock" treatment. All treatments were repeated within 1 week.

A sigmoidal response curve (3) was the appropriate function to describe electrolyte leakage from leaves of the three citrus cultivars as a function of treatment temperature (Fig. 1). The critical high temperature is defined as the inflection point of the curve (5, 9). There were no significant differences in the critical high temperature between rate and shock treatments for either cultivar. The critical temperatures for 'Glen' from the rate and shock treatments were estimated to be 54.3° ± 0.48° and 54.9° ± 0.41°, respectively, while those for 'Swingle' (1984) were 56.1° ± 0.42° and 55.6° ± 0.38°. There was no significant difference in the predicted critical temperature for 'Swingle' in 1984 and 1985 (data not shown). Although this study was conducted with excised leaves, we speculate that naturally occurring, brief daily temperature increases in the field do not increase heat tolerance for these two cultivars.

'Swingle' citrumelo leaves appear to be slightly more heat tolerant than 'Hamlin' orange and 'Glen' citrange (Fig. 1).

The canopy reached a maximum of 36.6°C when the air temperature was 37.7°. Therefore, it does not appear that 'Glen' citrange, 'Hamlin' orange, and 'Swingle' citrumelo leaves are normally subject to temperatures that would induce direct heat injury (7) under field conditions in Florida.

Literature Cited

1. Chen, H.-H., Z.-Y. Shen, and P.H. Li. 1982. Adaptability of crop plants to high temperature stress. Crop Sci. 22:719–725.
2. Furmanski, R.J. and R.W. Buescher. 1979. Influence of chilling on electrolyte leakage and internal conductivity of peach fruits. HortScience 14:167–168.
3. Ingram, D.L. 1985. Modeling high temperature and exposure time interactions on *Pittosporum tobira* root cell membrane thermostability. J. Amer. Soc. Hort. Sci. 110(4):470–473.
4. Ingram, D.L. 1986. Root cell membrane heat tolerance of two dwarf hollies. J. Amer. Soc. Hort. Sci. 111(2):270–272.
5. Ingram, D.L. and D.W. Buchanan. 1981. Measurement of direct heat injury of roots of three woody plants. HortScience 16(6):769–771.
6. Ingram, D.L. and D.W. Buchanan. 1984. Lethal high temperatures for roots of three citrus rootstocks. J. Amer. Soc. Hort. Sci. 109(2):189–193.
7. Levitt, J. 1980. Response of plants to environmental stresses. Vol. I. Chilling, freezing and high temperature stresses. Academic, New York.
8. Martineau, J.R., J.E. Specht, J.H. Williams, and C.Y. Sullivan. 1979. Temperature tolerance in soybeans. I. Evaluation of a technique for assessing cellular membrane thermostability. Crop Sci. 19:75–78.
9. Sullivan, C.Y. 1972. Mechanisms of heat and drought resistance in grain sorghum and methods of measurement, p. 247–264. In: N.G.P. Rao and L.R. House (eds.). Sorghum in the seventies. Oxford & I.B.H., New Delhi, India.

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Cold Hardiness of 'Midiron' and 'Tifgreen' Bermudagrass

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Abstract. **Electrolyte leakage and regrowth tests were used to estimate cold hardiness levels of field-grown 'Midiron' and 'Tifgreen' bermudagrass (*Cynodon dactylon* x *C. transvaalensis* crowns. The two procedures were in close agreement. 'Midiron' was hardier than 'Tifgreen' on all sampling dates. Greatest levels of freeze tolerance were −11°C for 'Midiron' and −7° for 'Tifgreen' during December and January. 'Midiron' was killed at −5° in early June while 'Tifgreen' had lost all freeze tolerance by this date. Although the electrolyte leakage procedure was rapid and required no greenhouse space, it was relatively difficult to set up and evaluate.**

Bermudagrass cultivars currently used throughout the northern boundaries of adaptation periodically sustain winter damage. As a result, selection of new cultivars with superior cold hardiness is an important breeding objective. The classical approach to selection for cold hardiness has been to evaluate field plots in the spring following a severe winter. Although this procedure probably gives the best indication of field response to low temperature stress, it can be very time-consuming. Extended evaluation periods become necessary when many years elapse between test winters. In addition, results are not reproducible, either in time or in regard to location due to the unpredictability of test winters.

Many workers have developed laboratory procedures to evaluate cold hardiness levels of turfgrasses and cereals. However, most of the early techniques provide only relative hardiness estimates, take considerable time and greenhouse space, or have limited application to crowns of warm-season grasses. Tests involving exposure to one treatment temperature in a freezer can provide information on relative hardiness if an appropriate temperature is chosen. Determination of absolute hardiness levels requires removing samples sequentially from a series of temperatures. This procedure does not lend itself to small tissue samples in an air-cooled chamber, since large temperature fluctuations can occur when the chamber is opened to remove samples. Refrigerated baths using ethylene glycol or alcohol vary less in temperature than air-cooled chambers. In addition, samples can be removed without disturbing experimental units of other treatment levels. This approach was used to evaluate freezing resistance of a variety of cool-season grasses (6, 8). The latter study also used the electrolyte leakage test to quantify plant responses. The turn-around time of the electrolyte leakage test was 2 days, compared to many weeks for regrowth tests.

Our objective was to determine whether the electrolyte leakage test could discern cold hardiness levels of bermudagrass crowns following exposure to freezing temperatures in a refrigerated bath. A procedure similar to one used by Rajashekar, et al. (8) was employed except the sensitivity of the test was increased by reducing the treatment temperature interval from 5° to 2°C. Results were compared on certain dates with regrowth tests of intact plants. Development of a rapid, reproducible testing procedure would facilitate evaluation of cold hardiness levels of several bermudagrass cultivars on an annual basis.

Two independent procedures were evaluated for determining cold hardiness levels of 'Midiron' and 'Tifgreen' bermudagrass (*Cynodon dactylon* (L.) Pers. x *C. transvaalensis* Burtt-Davy) from research plots in Stillwater, Okla. In the electrolyte leakage test, crowns were removed from the soil, thoroughly washed and placed in 25 × 150

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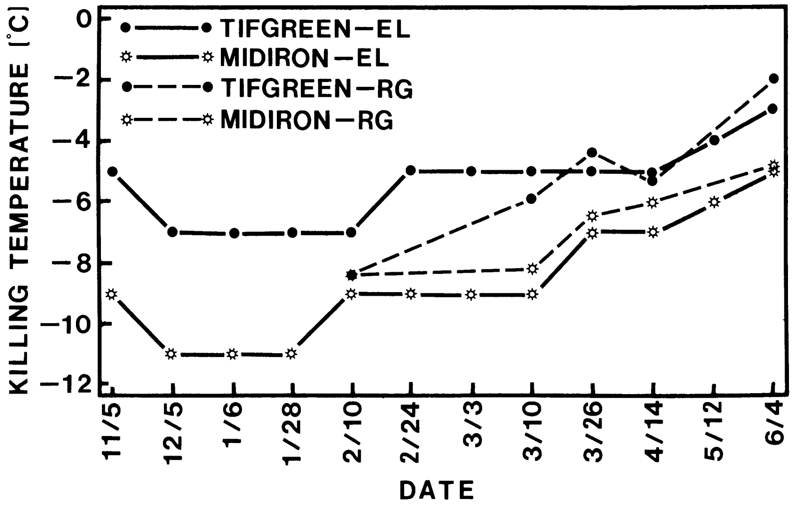

Fig. 1. Seasonal cold hardiness levels, expressed as killing temperature of 'Tifgreen' and 'Midiron' bermudagrass. Evaluations were made with electrolyte leakage (EL) and regrowth (RG) tests.

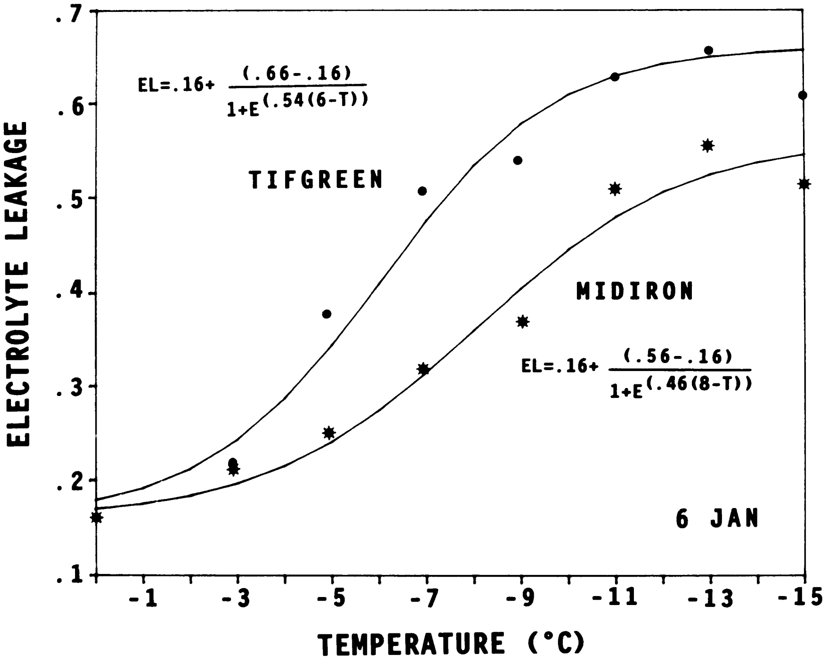

Fig. 2. Electrolyte leakage (EL) from 'Midiron' and 'Tifgreen' bermudagrass crowns following low temperature stress on 6 Jan. (E = 2.718; T = absolute value of treatment temperature).

mm test tubes. Tubes containing the crowns were submerged in a refrigerated bath with three replicates per treatment level. After equilibration at −3°C, chips of ice were dropped into the tubes to prevent excessive supercooling, and to ensure evaluation of freeze tolerance rather than avoidance. After being held overnight at −3°, samples were removed at 2° intervals while the bath was cooled at a rate of 2°/hr. Following slow thawing at 0°, 20 ml of distilled water was added to the tubes containing the crowns. The electrical conductivity of the water was measured 24 hr later (Model 35 conductance meter, Yellow Springs Instrument Co., Yellow Springs, Ohio). Samples were then heat-killed in an autoclave, and conductivity measurements taken following an additional 24 hr at room temperature. Response data were expressed as the ratio of the conductivity reading following exposure to freezing to the value after being heat-killed, thereby accounting for variation in crown mass. The killing temperature was determined as the warmest treatment level resulting in $\geq$40% loss of total electrolytes. This value was found to correspond most closely to the value with the greatest slope in a plot of electrolyte leakage vs. treatment temperature.

Response curves were fitted using the following model developed for electrolyte leakage data from heat stress studies (7): EL = $Y_{min} + (Y_{max} - Y_{min})/(1 + e^{k(Tm-T)})$ where EL = electrolyte leakage, Y_{min} = lower bound of EL, Y_{max} = upper bound of EL, Tm = temperature at the inflection point, k is a function of slope at Tm, and T = absolute value of treatment temperature. Parameters were estimated by the Gauss-Newton method of nonlinear regression (10).

In the second procedure, soil cylinders 60 mm in diameter and 80 mm in height containing intact plants were cooled at ≈1.5°C/hr in a low-temperature chamber capable of linear cooling rates (Model CEC 23, Rheem Scientific, Asheville, N.C.). Samples were removed at selected temperatures as measured by thermocouples inserted into each soil cylinder. The mass of the soil cylinders relative to isolated crowns reduced temperature disturbances of other experimental units when the chamber was opened to remove samples. The use of detachable plugs (Omega Scientific, Stamford, Conn.) in the thermocouple wires facilitated rapid removal of soil cylinders from the chamber since the thermocouple junctions typically froze into the soil. Samples were thawed at room temperature, then placed in 150-mm pots containing commercial soil mix and transferred to a greenhouse. Survival was judged by regrowth as determined visually 3 to 4 weeks later. The critical temperature in this test was taken as the coldest treatment temperature that retained at least one viable shoot.

Results of electrolyte leakage and regrowth tests were in close agreement, differing in hardiness estimates by 0.1° to 1.4°C (Figure 1). However, actual differences could have been somewhat larger since 2° temperature intervals were used. One-degree intervals should improve the accuracy of hardiness level estimates and may be more appropriate when comparing cultivars with small differences in hardiness.

'Midiron' was found to be hardier than 'Tifgreen' on all dates (except for the regrowth test on 10 Feb.), which is in agreement with previous observations of the two cultivars (1). The hardiness level of 'Tifgreen' in November and March was similar to values reported for 'U-3', 'Midway', and 'Westwood' in Missouri (3). Differences in killing temperatures of 2° to 4°C were estimated by the electrolyte leakage test while regrowth indicated hardiness differences of 0° to 2.3° between cultivars. 'Tifgreen' did not permit this comparison on 4 June because the warmest subfreezing temperature treatment from both tests killed all of the samples.

Both 'Midiron' and 'Tifgreen' were within 2°C of their maximum hardiness on 5 Nov., the first sampling date. The period of greatest freeze tolerance was December and January for both cultivars. 'Tifgreen' retained hardiness to about −5° through mid-April, losing all freeze tolerance by 4 June. 'Midiron' was killed at about −7° in mid-April and retained freeze tolerance to −5° on 4 June. We do not know if or when 'Midiron' loses the capacity to tolerate ice formation within the tissues. Freeze tolerance was observed subsequent to growth of shoots in the spring.

These results are valid only for the acclimating conditions to which the plants were exposed. Other locations and/or years may result in different hardiness levels. These experiments were designed to detect differences in freeze tolerance. Samples were nucleated with ice to prevent supercooling, since damage in winter wheat was increased when crowns supercooled to temperatures

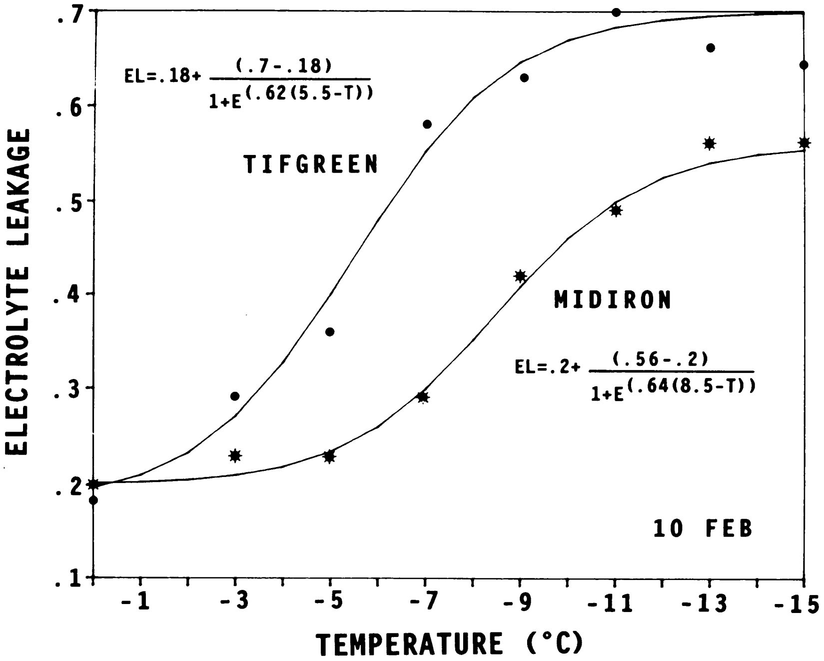

Fig. 3. Electrolyte leakage (EL) from 'Midiron' and 'Tifgreen' bermudagrass crowns following low temperature stress on 10 Feb. (E = 2.718; T = absolute value of treatment temperature).

≤3°C before freezing (5). Long equilibration periods for initial freezing and slow cooling rates allowed tissues to come to equilibrium with the stress. Any survival features in the field, such as deeply buried organs, that would promote alleviation of low temperature stress by temperature buffering, would not be detected.

The relationship between electrolyte leakage and treatment temperature was sigmoidal in Kentucky bluegrass leaves, while crowns yielded a nearly linear relationship (8). Similarly, a one-third reduction in percentage survival of 'Meyer' zoysia occurred over a span of 5.5°C (9). Our results generally suggest a gradual transition from undamaged to killed tissues over the span of several degrees (Figs. 2 and 3). Undoubtedly, a study with a broad range of treatment temperatures with large intervals between treatment levels favors sigmoidal curves at the expense of differentiating small differences in cold hardiness. The procedure could be improved if the moisture status of the crowns were determined. Extremes in tissue hydration have been shown to affect the hardiness level of dogwood stems (2), and crowns of Kentucky bluegrass (6). Gusta and Fowler (4) found a close correlation between water content and killing temperature in deacclimating cereal crowns. Since plant material for our study was gathered from plots a few meters apart, it was likely that crowns were exposed to similar soil moisture contents.

The electrolyte leakage test was able to measure cold hardiness levels of 'Tifgreen' and 'Midiron' bermudagrass crowns on a seasonal basis. The relative hardiness of the two cultivars agreed with previous field observations. In addition, the test corresponded closely to another independent estimate of freeze tolerance. The short turn-around time and lack of need for greenhouse space are advantages of the electrolyte leakage procedure. The major drawback may be the intensive labor necessary to excise and thoroughly wash crowns from a large number of cultivars.

Literature Cited

1. Beard, J.B., S.M. Batten, and G. Pittman. 1980. The comparative low temperature hardiness of 19 bermudagrasses. Texas Agr. Expt. Sta. Prog. Rpt. 3835.
2. Chen, P.M., P.H. Li, and M.J. Burke. 1977. Induction of frost hardiness in stem cortical tissues of *Cornus stolonifera* Michx. by water stress I. Unfrozen water in cortical tissues and water status in plants and soil. Plant Physiol. 59:236–239.
3. 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.
4. Gusta, L.V. and D.B. Fowler. 1976. Effects of temperature on dehardening and rehardening of winter cereals. Can. J. Plant Sci. 56:673–678.
5. Gusta, L.V. and D.B. Fowler. 1977. Factors affecting the cold survival of winter cereals. Can. J. Plant Sci. 57:213–219.
6. Gusta, L.V., J.D. Butler, C. Rajashekar, and M.J. Burke. 1980. Freezing resistance of perennial turfgrasses. HortScience 15:494–496.
7. Ingram, D.L. 1985. Modeling high temperature and exposure time interactions on *Pittosporum tobira* root cell membrane thermostabilitay. J. Amer. Soc. Hort. Sci. 110:470–473.
8. Rajashekar, C., D. Tao, and P.H. Li. 1983. Freezing resistance and cold acclimation in turfgrasses. HortScience 18:91–93.
9. Rogers, R.A., J.H. Dunn, and C.J. Nelson. 1975. Cold hardening and carbohydrate composition of Meyer zoysia. Agron. J. 67:836–838.
10. SAS Institute Inc. 1985. The NLIN procedure, p. 575–614. In: SAS user's guide: statistics. Version 5 edition. SAS Institute Inc., Cary, N.C.