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Freeze-Stress Resistance

How freeze-stress resistance in perennial ryegrass relates to turfgrass performance

By J. S. Ebdon, Ph. D.

Each winter in New England and the northern United States, substantial turf losses occur due to injury from freezing temperatures. Turf injury and losses that result from freezing temperatures can have an economic and environmental impact on the functional quality and aesthetic value of turf areas. Turf loss results in increased weed pressure and herbicide cost, increased soil erosion, decreased use and, in the end, the need for costly and extensive re-establishment (Dipaola and Beard, 1992).

Turfgrass species and varieties vary considerably in their tolerance to freezing stress (Gusta et al., 1980). Perennial ryegrass (Lolium perenne L.) has been reported to have the poorest low temperature tolerance among cool-season turfgrass species (Beard, 1973). However, cultivars of perennial ryegrass can vary widely in their lethal killing temperatures (LT_{50}), ranging from -5 to -15 °C (Gusta et al., 1980).

Despite having poor low temperature tolerance, perennial ryegrass is still one of the most important and widely used species in the northern United States (Meyer and Funk, 1989; Watson et al., 1992). Its ability to establish quickly makes it a popular choice of turf managers for overseeding fairways, institutional grounds, parks, home lawns and in lawn care operations. It is expected that the popularity of perennial ryegrass will continue to increase in the northeast and elsewhere with the release of new and improved cultivars.

Turfgrass freezing stress

Turfgrass freezing stress occurs at 0 °C (32 °F) and colder temperatures. Injury to turfgrass due to freezing temperatures involves the formation of ice crystals in and around the cells of the regenerative region of the plant (Beard, 1973; Rossi, 1997). The regenerative region of a turfgrass plant, also known as the crown, is the region that includes the stem apex, the unelongated internodes and the lower nodes from which the adventitious roots are initiated (Hull, 2000). Since adventitious roots, lateral shoots (tillers, rhizomes, and stolons) and leaves all initiate from this region, the crown tissue is considered the most vital portion of a turfgrass plant (Beard, 1973; Hull, 2000).

If temperatures drop quickly, intracellular freezing will occur in tissues, especially those having high tissue hydration levels. The ice crystals cause a mechanical disruption to the cell membranes that result in death of the tissue. The lysis of cells with the release of cell contents from this type of injury can be measured in the laboratory using electrolyte leakage methods. This approach is effective in identifying injury at the cellular level.

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An alternative approach effective in assessing actual survival from freezing injury is to use whole plants and freeze-shock recovery methods.

Ability of turfgrass to survive freezing stress is a function of the severity of the injury and its location within the crown.

The ability of turfgrass to survive freezing stress is a function of the severity and location of the injury within the crown. If a sufficient number of cells within the crown of the turfgrass plant are injured, the grass may not recover. Whole-plant regrowth and recovery studies following exposure of plant samples to low-temperature stress is the most effective means of evaluating freezing stress tolerance and is often used to assess low temperature survival. Whole-plant survival of bunch-type grasses such as perennial ryegrass depends on the production of tillers.

Improved survival of hardened perennial ryegrass depends on the ability to establish viable regrowth from lateral tiller buds (Eagles et al., 1993).

It has been reported that the lower portion of annual bluegrass crowns are more likely to be injured, due to freezing stress, than the upper portion (Beard and Olien, 1963; Beard, 1973). The lower apical meristem responsible for root initiation can be injured more easily than the upper apical meristem (Beard, 1973; Olien and Marchetti, 1976). This injury can be exacerbated by desiccation in early spring when transpiration resulting from warming temperatures and resumed growth exceeds the water uptake capability of the injured roots.

For some grass species, survival to freezing stress can be limited by the tolerance of a relatively small number of cells in the basal region of the crown (transition zone between the root and shoot), rather than the apical meristem (shoot apices and lateral buds) (Shibata and Shimada, 1986; Tanino and McKersie, 1985). Different regions of the crown are killed at different temperatures (Eagles et al., 1993) because of differential hardening within the crown itself. Recovery is still possible providing critical crown regions important in regrowth remain viable.

Freeze-stress tolerance, quality

Freezing stress is a major factor limiting the adaptation of turfgrass to northern climates (Beard, 1973). For species with marginal low-temperature hardiness, such as perennial ryegrass, this may be an especially important limitation to growth, function and overall quality of the turf.

Turfgrass quality is a subjective measurement of aesthetic appeal and functional value, and includes components such as color, shoot density, uniformity and texture (Turgeon, 1980). Therefore, shoot density (tiller density), which is an important component of turfgrass quality, is directly affected by direct low-temperature kill.

In perennial ryegrass, whole-plant survival to freeze-stress temperatures depends on tiller production for regrowth. Superior tillering capacity could have a beneficial effect on the recovery from low-temperature stress because more tiller buds are available for regrowth, thus influencing winter survival. Superior turfgrass performers could have improved freeze-stress recovery and survival because of more profuse tillering compared to poor performers. Accordingly, there is a potential link between turfgrass quality and the capacity to recover from freeze-stress injury.

The goal of turfgrass managers is to select grasses that are well adapted to environmental stresses that limit turfgrass growth at their location. To that end, a study was initiated at the University of Massachusetts to compare the freezing-temperature thresholds for survival in cultivars of perennial ryegrass representing contrasting qualities. The study emphasized low-temperature tolerance and its relative contribution to turfgrass quality when targeting turfgrass to northern climates.

Plant material selection

The criteria for cultivar selection was based on the relative ranking (top and bottom cul-

The popularity of perennial ryegrass will continue to increase in the Northeast and elsewhere with the release of improved cultivars.

tivars) from the most recent (1997) National Turfgrass Evaluation Program (NTEP) field trial conducted at the Maine (Orono) location (the most northern NTEP location in New England) (USDA, 1997). Ten perennial ryegrass cultivars representing diverse quality types (5-high and 5-low ranking cultivars) were chosen (Table 1). Cultivars LRF-94-C8, Palmer III, Prelude III, Repell III and Top Hat represented high (superior) performers, and DSV NA 9401, DSV NA 9402, Linn, Pennfine and SR-4010 were selected to represent the low (poor) performing varieties.

The average January air temperature for Maine during the period from 1994 to 1998 (the corresponding NTEP evaluation period on which the cultivar selection was based) was -10.3 °C (+13.5 °F) (Table 2, NRCC report).

Air temperature is less important to crown survival than soil temperature since crowns are located near or below the soil surface and are protected by the warmer soil temperatures (Beard, 1973). The air temperature data summarized in Table 2 indicates, however, that the average temperature for January in Maine is within the lethal temperature range of –5 to –15 °C for some perennial ryegrass cultivars (Gusta et al., 1980). There was considerable range in January temperatures (and the potential for freezing-stress injury) between northern New England states (Maine, New Hampshire and Vermont) and southern New England (Massachusetts, Connecticut and Rhode Island).

Acclimation

Cultivars were established from seed and were sown (September 23, 1998) in 2-in. diameter by 7-in. deep pots filled with a commercial planting mix consisting of peat, perlite and vermiculite. Cultivars representing "LOW" and "HIGH" performance types were evaluated under two environments representing "Acclimated" and "Non-Acclimated" tissue.

On December 9, 1998, container plants were transferred following a 10-week establishment period in a heated greenhouse to the field and placed in a cold frame in a covered but open-ended polyhouse at the University of Massachusetts Turfgrass Research Farm in South Deerfield, MA. Containers were placed directly on the soil surface as close to each other as possible. The plants were kept in the cold frame for the remainder of the fall season into winter in order to simulate field acclimation (physiological hardening) conditions as closely as possible. Non-acclimated plants were kept in the heated greenhouse (70 °F) during the same period.

TABLE 1. RELATIVE RANKING AT THE MAINE-ORONO NTEP LOCATION

(1=highest, 96=lowest turfgrass quality ranking)

Cultivar	Rank
High-performance group	
LRF-94-C8	6.0
Palmer III	3.0
Prelude III	1.5
Repell III	1.5
Top Hat	4.0
Low-performance group	
DSV NA 9401	93.0
DSV NA 9402	89.5
Linn	95.5
Pennfine	94.5
SR-4010	95.5

Crown and root-zone temperatures were monitored. Figure 1 shows the mean daily soil temperature vs. the plant container media temperature at the surface and 2.5-cm (1-in.) into the soil/media. Note that the average surface temperature of the plant media (which is in intimate contact

TABLE 2. AVERAGE JANUARY AIR TEMPERATURE (°C) FROM 1994 THROUGH 1998 FOR THE NEW ENGLAND REGION. FROM THE NORTHEAST REGIONAL CLIMATE CENTER.

State	1994	1995	1996	1997	1998	Avg.
ME	-15.6*	-7.2	-10.3	-10.1	-8.1	-10.3
VT	-14.3	-4.2	-9.1	-8.5	-5.3	-8.3
NH	-12.6	-3.7	-7.4	-7.8	-4.8	-7.3
MA	-7.9	+0.1	-3.9	-3.4	-0.9	-3.2
CT	-7.5	+0.1	-4.1	-3.2	+0.3	-2.9
RI	-4.7	+2.1	-1.7	-1.2	+1.8	-0.7

* Lowest average temperature for January ever recorded.

with crown tissues) dropped to a low of -12.6 °C (+9.3 °F) by January 15, which is lower than the temperature threshold for survival (LT50) of some perennial ryegrass cultivars. Average soil temperatures were considerably warmer and less variable than container media.

Freeze-shock recovery (survival)

Freezing shock and subsequent recovery of plant material was evaluated by submitting container plants to a range of 11 decreasing treatment temperatures consisting of a non-frozen control (+5 °C), and 10 freeze-stress temperatures: -3, -5, -7, -9, -11, -13, -15,-17, -19, and -21 °C.

Treatment temperatures were applied using a programmable freezer. After temperature exposure, the plant material was removed from the freezer and assessed for survival as a percentage of viable-green shoots. Plant samples were planted in cell trays and placed in the greenhouse for a four-week recovery period.

Plants with any green surviving tissues or any new growth from even one shoot were counted as survivors. All others were considered as having been killed by the treatment temperature. The temperature at which 50% of the crown tissue survived based on regrowth recovery was determined statistically and expressed as LT50.

Effects of acclimation on low-temperature hardiness

Cold acclimation (hardiness) involves physiological changes within the plant. As fall soil temperatures approach 45 °F, turfgrass shoot growth slows and eventually stops. Carbohydrate levels increase and tissue (crown) hydration levels decrease, resulting in the tissue achieving maximum low-temperature hardiness (Beard, 1973; Levitt, 1980). A period of 3 to 4 weeks of average daily air and soil temperatures between 34 to 45 °F are optimum to harden cool-season turfgrass (Beard, 1973).

Significant acclimation effects of perennial ryegrass were observed in our studies. Acclimated cultivars (AC, cold framed conditioned plants) of perennial ryegrass exhibited greater freeze-stress tolerance than non-acclimated plants (NA, greenhouse conditioned plants). AC plants had significantly lower (more negative) LT50 estimates than NA plants based on whole-plant survival evaluations (Table 3). AC tissues had a mean lethal killing temperature (LT50) of -8.4 °C (+16.9 °F) compared to NA plants that had a mean LT50 of only -1.8 °C (+28.8 °F). The range in cultivar LT50 values for AC plants (-3.0 to -14.7 °C) was substantially greater than observed for NA plants (+3.9 to -4.2 °C).

After four weeks of regrowth, a visual count of survivors of AC plants showed that some plants from a few cultivars had survived temperatures as low as -21 °C (-5.8 °F). However, there were no survivors of NA plants exposed to temperatures lower than -7 °C (+19.4 °F). The 25 °F lower killing temperature for AC plants compared to NA plants indicate the importance of cold acclimation to low temperature survival. These results also suggest that perennial ryegrass maintained in an unheated polyhouse had sufficient time to adjust (acclimate) to freezing temperatures after 2 1/2 weeks in the cold frame (Fig. 1) and therefore tested the ability of ryegrass to acclimate quickly.

Difference in hardiness between performance groups

Perennial ryegrass cultivars that consistently performed well (ranking in the top 5%) in turfgrass variety trials at the Maine-Orono NTEP location were distinctly different in freezing survival from their poor-performing counterparts (cultivars ranking in the bottom 5%). Based on LT50 estimates, the high-performance cultivars (LRF-94-C8, Palmer III, Prelude III, Repell III, and Top Hat) had superior whole-plant survival following exposure to freezing treatments compared to the low-performance group (DSV NA 9401, DSV NA 9402, Linn, Pennfine, and SR-4010). AC plants for high-performance cultivars were able to survive lower temperatures indicated by a lower (more negative) mean LT50 estimate (-10.9 °C or +12.4 °F) compared to the low-cultivar group (mean LT50 of -6.0 °C or +21.2 °F, Table 3).

Visual differences between cultivar groups in shoot density following low temperature exposure were striking after four weeks of recovery in the greenhouse (Fig. 2). Such losses in shoot density that were observed with low-performance cultivars with decreasing temperature have obvious implications for these ryegrasses under the extreme low temperatures typical of the Maine-Orono location (Table 2).

The mechanism of freeze-stress injury was directly related to injury to cell membranes (Fig. 3). Specifically, high-performance cultivars experienced significantly less leakage of cell electrolytes (an indication of less cell membrane disruption) compared to poor-performing ryegrasses.

Differences between performance types in whole-plant survival were closely associated with injury at the cellular level. In general, hardened (acclimated) ryegrasses and high-performance cultivars were less sensitive to freezing-injury with decreasing tem-

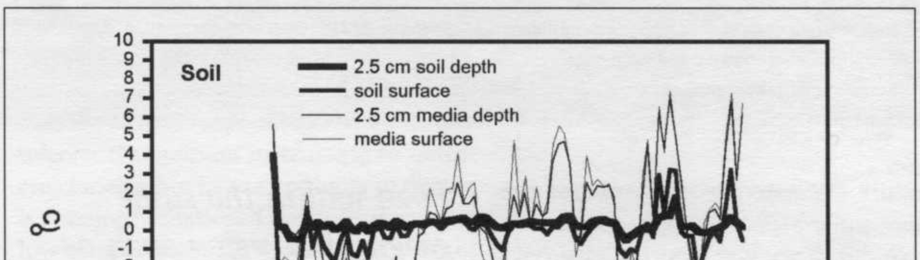

Figure 1. Mean daily soil temperature comparison between actual soil and plant container media at various depths during the experimental period.

TABLE 3. COMPARISON OF LT_{50} ESTIMATES FOR HIGH AND LOW PERFORMANCE GROUPS OF PERENNIAL RYEGRASS.

	LT50 (°C) by environment	
Cultivar	Acclimated (AC)	Non-acclimated (NA)
High performance group		
LRF-94-C8	14.7	-3.7
Palmer III	-10.0	-2.9
Prelude III	-9.3	-4.2
Repell III	-10.2	-2.7
Top Hat	-10.1	-1.8
Mean-high group	-10.9	-3.0
Low performance group		
DSV NA 9401	-3.9	-2.2
DSV NA 9402	-8.3	-1.2
Linn	-3.0	+3.9
Pennfine	-11.8	-2.7
SR-4010	-3.0	-1.1
Mean-low group	-6.0	-0.7
Mean by environment	-8.4	-1.8
Significance (p-value)		
Mean high vs. low†	0.05	NS
Mean AC vs. NA‡	0.001	

†Comparison between group means within environment (AC and NA).
‡Comparison between AC and NA means.
NS=not statistically significant.

perature compared to nonhardened ryegrasses and low-performance cultivars.

No difference was detected in survival (or electrolyte leakage) between high- and low-performance groups for NA plants. Therefore, survival differences between contrasting turf quality types were only detected when cultivars were allowed to adjust to low temperature through acclimation.

Freezing stress is an obvious limiting factor in adapting to Maine winters, and improved low-temperature survival is an important criteria for turfgrass managers to consider when selecting perennial ryegrass genotypes for northern environments.

Research has shown that winning cultivars from variety trials conducted in one geographic region (e. g., New England) may not necessarily perform well in other regions where cool-season turfgrass is adapted (USDA, 1997). Even within the

Even within the same geographic region such as New England, significant climatic differences in temperature can exist that limit turfgrass survival and functional quality.

same geographic region such as New England, significant climatic differences in temperature can exist (Table 2) that can limit turfgrass survival and functional quality. For example, the winning genotypes (top 5%) from the Maine NTEP location (Table 1) are different from the winning genotypes from the Rhode Island NTEP location. Use caution when extrapolating NTEP results from outside your own geographic zone or when extrapolating our results.

Important considerations

It is important to recognize that turfgrass quality (the basis for selection of high- and low-performance groups used in this study) is an integration of several components described earlier. The loss in shoot density caused by direct low-temperature kill is one of many stresses operating during the year that may limit turfgrass growth and quality. Superior low-temperature survival alone does not necessarily equate to superior turfgrass performance.

The cultivar Pennfine, a low performance type (Table 1), possessed superior low-temperature survival characteristics (LT50) similar to high-performance cultivars (Table 3). Pennfine's poor (low) rating in variety trials is in part due to its susceptibility to leaf spot (Bipolaris spp.) and brown patch (Rhizoctonia solani) disease (USDA, 1997). Consequently, it shares some of the attributes in common with high-performance cultivars (superior low-temperature tolerance) important in adapting to northern climates, but its susceptibility to disease is a major limitation. Therefore, improved low-temperature survival is just one of many considerations in selecting and breeding improved turfgrass varieties.

Other important turf-forming properties include improved disease and insect resistance, enhanced environmental stress tolerance, and reduced mowing, fertilizer, irrigation and pesticide requirements (Meyer and Funk, 1989).

Eagles et al. (1993) suggested that the release of apical dominance after the death of the main apex was the method of recovery in perennial ryegrass. The main apex of perennial ryegrass is killed at higher temperatures compared to lateral tiller buds. They also reasoned that profuse tillering could have a beneficial effect on recovery by providing more tiller buds as potential sites for regrowth. Shoot density measurements obtained from NTEP reports (USDA, 1997) indicate the high-performance group had significantly more shoots compared to the low group. This implies the potential for greater tillering and winter survival with high-performance cultivars as suggested by Eagles et al. (1993).

Freezing-stress resistance is a complex physiological process, complicated by inherent genetic differences between species and cultivars (Table 3); physiological alterations in freeze-stress resistance (hardening or dehardening) caused by management inputs (mowing height, nitrogen and potassium levels, thatch levels, plant growth regulator use and timing, and drainage considerations); and influenced by climatic differences that can exist from region-to-region and year-to-year (Table 2), affecting the intensity of low-temperature stress and the physiology of hardening or dehardening.

In the end, superior low-temperature survival is closely linked to improved performance and function. It is important to appreciate and understand how to effectively manage those species having poor or

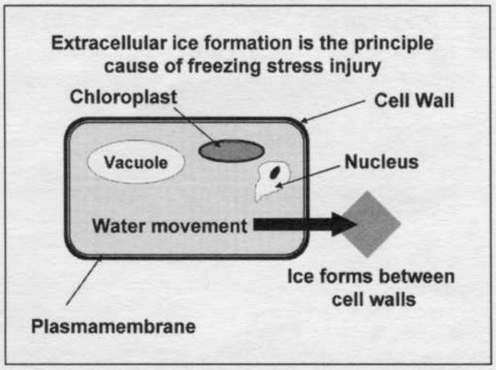

Figure 3. Mechanical disruption of cell membranes caused by water movement out of cells associated with the formation of extracellular ice (between cell walls). Injured (leaky) cells can be measured using conductivity bridges; higher electrical conductivity equates to greater injury (disruption of plasmamembrane).

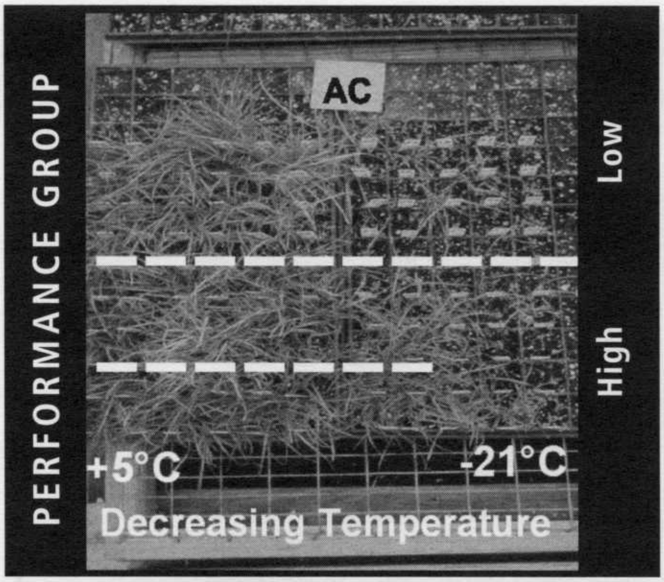

Figure 2. Regrowth survival of acclimated perennial ryegrass (5-low performance cultivars, upper half; 5-high performance cultivars, lower half) at 4 weeks following exposure to freezing temperatures. Note the higher shoot density (recovery) with high-performance cultivars under extreme low temperature.

marginal freeze-stress resistance such as perennial ryegrass, tall fescue and annual bluegrass to ensure maximum survival and functional quality in northern zones. The complex nature of low-temperature hardiness is not fully understood. Research is still needed to better understand the relationship between various management practices on acclimation (and de-acclimation) for those species and cultivars susceptible to low-temperature kill.

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