A PRACTICAL RESEARCH DIGEST FOR TURF MANAGERS

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Physiology of Turfgrass Freezing Stress Injury

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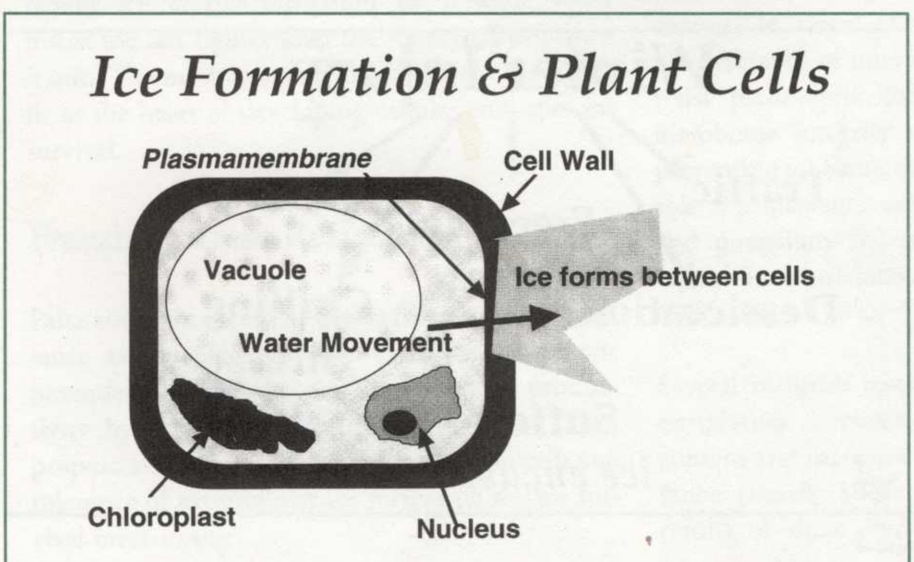
Through the years, golf course management has been impacted by the introduction of various technologies. Technological advances have enabled golf course superintendents to maintain higher quality turf and playing conditions than could be expected if technology was unavailable. Does it follow then that technology gives us control?

The answer is different depending on the context in which the question is asked. Surely, mechanical and chemical technology have provided useful tools for achieving superior putting surfaces. Still, when it comes to the various aspects of winter injury on northern golf turf, the last few winters have demonstrated the harsh reality of how precious little we control.

Recent devastating losses from winter injury have revitalized interest in this otherwise neglected area, as evidenced by the number of articles in popular trade magazines, conference topics and university research programs. Minimizing turf loss in winter requires improved comprehension of the



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freezing tolerance processes at work. We simply cannot influence what we do not understand.

Overview

Each year, throughout the northern United States, thousands of acres of turf are lost to what has been termed "winter injury." Ironically, estimates from industry surveys indicate 35-75% of all energy inputs to turf management are preparations for and recovery from winter. Nevertheless, substantial turf loss occurred following the 1992-93 winter in the midwestern U.S. and the 1993-94 winter in the northeastern U.S.. Extensive turf loss has significant environmental and economic consequences on the functional and aesthetic quality of recreational turf areas. Turf loss from winter injury, most evident in the spring, results in increased weed encroachment, greater soil erosion, and often requires energy intensive re-establishment procedures to restore the environmental benefits of a contiguous and healthy grass cover.

Research is needed to answer basic questions concerning the environmental and physiological conditions resulting in freezing stress injury to cool-season turfgrasses. The lack of information regarding turfgrass was evident in a recent review of low-temperature stress. In that review, 85% of the literature cited represented cereal grain research that can only be extrapolated cautiously to turfgrass systems (DiPaola and Beard, 1992). Annual crops might avoid stress periods through annual planting and harvesting practices; however, perennial turf must suffer injury, enter dormancy, or otherwise survive the stress of low-temperatures. A more complete understanding of how freezing affects turfgrass is essential to the development of winter hardy plants and more energy efficient and environmentally sound management systems less reliant on pesticides.

Turfgrass Freezing Stress

Turfgrasses are injured or killed during winter in northern climates as a result of the singlular or combined effects of freezing stress, traffic, desiccation, soil

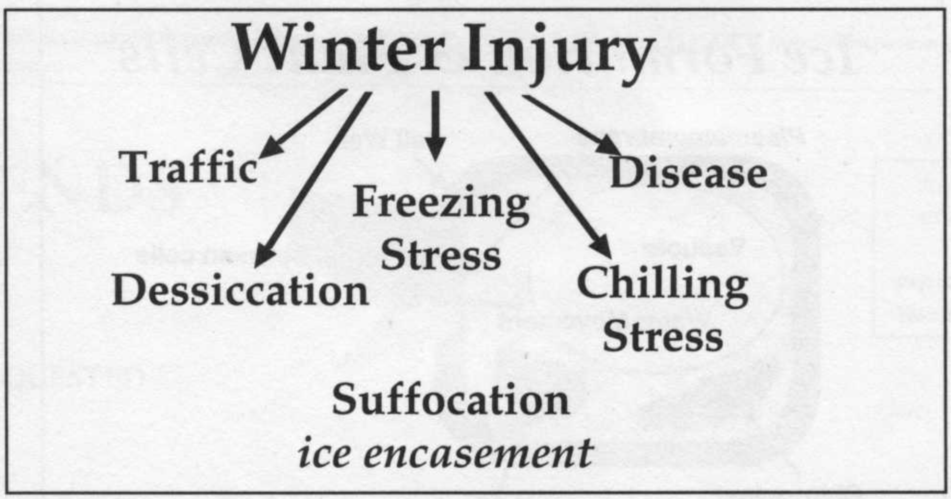

Figure 1

frost-heaving and low-temperature fungi (Beard, 1973)(**Figure 1**). Despite the multitude of interactive low-temperature stresses, freezing stress is thought to be the major factor affecting the survival of turfgrasses in the northern U.S. (DiPaola and Beard, 1992).

Turfgrass injury from freezing stress is directly related to how, where, and if ice forms in cells of the turfgrass stem apex (a.k.a. crown), the regenerative region of the grass plant that overwinters. Specifically, if temperatures drop rapidly and water is available for freezing **inside** a plant cell, that cell will be killed. If several cells in the crown die, the grass plant may not be able to recover. This direct form of freezing injury is thought to be rare, because temperatures generally decline slowly (between 1 to 2°C/ hour), allowing apical cells time to adapt.

The more common scenario is for ice to form **between** plant cells. As ice crystals form, they draw water molecules from inside the cells to expand the size of the crystals. As water is drawn from the cells, they become dehydrated. Dehydration causes a number of problems for cells, not the least of which is membrane function that allows even more water to flow out. Dehydration can cause the degradation of other cellular components, which also can result in the death of a cell. Again, if enough cells in the crown are killed, the grass will not recover. Plants utilize various mechanisms to reduce ice crystal formation by holding water inside the cell tighter than the ice crystal can draw it out. The mechanisms of freezing stress resistance lie at the heart of developing cellular strategies for survival.

Freezing Stress Resistance

Palta and Simon (1993) define freezing stress resistance as the plant's ability to realize its genetic potential for growth, development and productivity by surviving freezing temperatures. They propose avoidance of ice formation within cells and tolerance of extracellular ice formation as two survival mechanisms.

Avoidance. An interesting mechanism of injury avoidance demonstrated by insects, some mammals and several woody species is deep supercooling (Lee, 1991; Costanzo et al., 1992; Rajashekar 1988). It seems reasonable that the accumulation of sugars inside cells during cold acclimation could, to a certain extent, lower the freezing point and avoid injury by allowing cells to supercool. However, several researchers have observed only small (<4° C) depressions in the freezing point, and by itself, supercooling is not viewed to be very important in freeze stress avoidance in turfgrasses (Williams, 1980; Levitt, 1978 & 1980).

Tolerance. Levitt (1980) states that extracellular ice formation resulting in cell plasmolysis and the subsequent reduction of cell volume past a critical value, is the principle, if not the sole cause of freezing stress injury. Theoretically, if a semipermeable membrane separates two compartments differing only in solute concentration (temperatures are constant), then only solvent (i.e. water) moves through the membrane from the solution in the less concentrated compartment into the more concentrated compartment. When the compartments reach equilibrium, net flow of water between them ceases. Plant cells with high solute levels in the cytoplasm, differentially permeable membranes, and relatively rigid cell walls, would limit net water movement from the interior toward ice crystals forming extracellularly. Palta and Li (1980) have demonstrated alteration in membrane function by incipient freezing injury without changes in water permeability. This allows the maintainance of internal cell pressure which could resist plasmolysis and thus aid in maintaining membrane integrity under freezing stress, while preventing ice formation within the cells. Still, the role of solutes such as non-structural carbohydrates and potassium (K) nutrition in regulating frost injury remains inconclusive (Beard and Rieke, 1966; Olien 1984).

Several turfgrass researchers have demonstrated a correlation between reduced crown moisture content and increased turfgrass freezing stress resistance (Beard, 1966; Gusta et al., 1980). The results of these studies were presented as LT50 values unable to detect small but important differences in freezing resistance (Brule-Babel and Fowler, 1989) (**Figure 2**). Clearly, an important tolerance mechanism involves a reduction in crown moisture levels coinciding with acclimation.

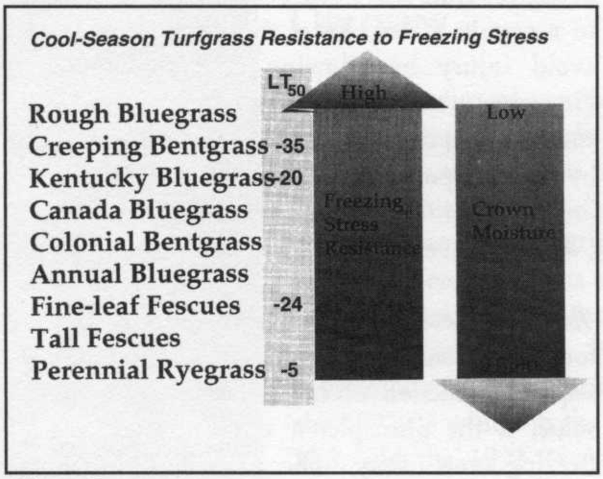

Figure 2

Cold Acclimation. A plant's capacity to cold acclimate and later to deacclimate has long been considered a significant factor determining freezing resistance (Carroll, 1943; Gay and Eagles, 1991; Fry et al., 1993; Palta and Simon, 1993). It has been suggested that some turfgrasses begin to cold acclimate during summer months and reach peak acclimation during mid-winter (White, 1981). As winter progresses, several physiological alterations occur during incipient freeze-thaw cycles (characteristic of late-winter, early-spring conditions) such as changes in non-structural carbohydrate status, hormone levels (ABA, GA) and crown moisture content (Levitt, 1980). An abundance of observational information reported in popular turfgrass literature suggests this transitional period from winter to spring is the critical stage when considering winter kill.

The Transitional Period. It has become apparent over the last several years that the transitional period between winter and spring, characterized by fluctuating freezing and thawing events, is critical to understanding plant death as a result of freezing stress (Roberts, 1993). During this time before plant energy reserves become low, the plant responds to warming temperatures by stimulating growth. When growth is stimulated, several physiological changes occur--the most significant being the hydration of tissue. The driving force for growth is the influx of water into plant cells. Unfortunately, as the crown becomes hydrated and resumes growth it becomes more susceptible to freezing injury than while in a hardened state, because more free water is available for freezing.

We must be clear that tissue hydrates when it begins to grow. This may occur in low, poorly-drained areas as a result of standing water that is warmed by solar radiation. Once the water warms, heat is transferred to the soil and grass plants, growth is stimulated, and water is taken up. It is important to note, however, that since crown hydration will occur anywhere growth is stimulated and water is available for uptake, injury during transitional periods may be more likely in poorly-drained areas, but it is not confined to them.

Maximizing Freezing Stress Tolerance

The question remains whether or not we have the technology to protect turfgrasses from freezing stress injury. Maximizing freezing stress tolerance must focus on several physiological conditions including: crown moisture, acclimation-deacclimation mechanisms, cell membrane integrity and energy storage. Understanding the contributions and interaction of each of these areas to the overall freezing stress response could provide information necessary to develop management strategies to minimize injury.

Energy Storage

Turfgrass is not entirely dormant during winter; instead, its physiology changes, much as our physiology is altered when we sleep (**Figure 3**). Since the plants continue to respire and utilize their energy supply as they overwinter, entering winter with high levels of stored energy could provide turfgrasses with several protective strategies.

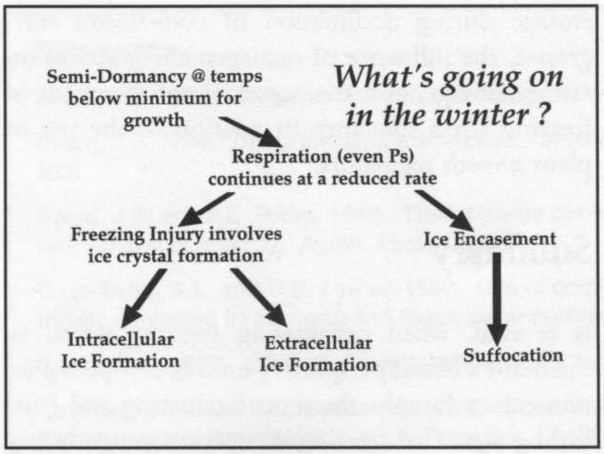

Figure 3

Warming temperatures during the transitional period are thought to stimulate growth, which sets grasses up for the kill. Not all turfgrasses deacclimate (or deharden) under the same temperature regimes (Gay and Eagles, 1991). It seems reasonable that grasses might deacclimate when energy storage is below some critical level and there is a need to produce energy for survival. It is also reasonable that elevated energy storage levels during acclimation might make the plant less likely to deacclimate early in the spring, because energy storage would be above the critical level. Our research at the University of Wisconsin-Madison is currently quantifying the critical energy level for several cool-season turfgrasses, specifically annual bluegrass.

Energy Storage and Cellular Water

As mentioned previously, ice crystal formation between cells exerts a draw on water inside cells, resulting in cell dehydration, so plants reduce cellular water levels during acclimation and subsequent freezing conditions. Still, when temperatures warm during the transitional period (winter to spring), cells rehydrate.

Remember from high school chemistry how water (or any liquid) moves from a higher concentration to a lower concentration? This process explains how water moves out of plant cells to form an ice crystal. The cell membrane prevents solutes, like energy sources (sugars and fructans), from leaving the cell and allows water (a liquid) to pass through, which is why we call it a semi-permeable membrane.

As the ice crystal forms it has a lower concentration of free water than exists inside the cell, and water moves out of the cell to enlarge the crystal. Maximizing the concentration of solutes in the cell could reduce the concentration of water in the cell. This reduced concentration would prevent the water from passing through the membrane to enlarge ice crystals. The cell would retain water as hydrated solutes and survive, because free water in the cell would be almost absent.

Management to Enhance Energy Storage

Several researchers working with cereal grasses (wheat, oats, barley) have correlated freezing stress tolerance with energy storage levels--increased energy storage in the grasses results in greater freezing stress tolerance. If cereal grasses are not bred with the ability to store high levels of energy, cereal production strategies to maximize energy are not practical; however, turfgrass management provides several potential strategies to enhance energy storage.

As with cereal grasses, turfgrass managers might start with plant material that has demonstrated good freezing stress tolerance. It might be possible through primary cultural practices (mowing, fertilization and irrigation) to maximize energy storage during acclimation.

Several researchers have investigated the role of potassium (K) in freezing stress tolerance; however, the role of K in energy production and storage remains unclear. For example, does the accumulation of K in the cell act as a solute aiding the ability of the cell to bind water during ice crystal formation? In general, information has been conflicting and often inconclusive.

Growth Regulation

The introduction of plant growth regulators in turfgrass management was motivated by a desire to reduce mowing requirements. Generally, plant growth regulators reduce growth by blocking the production of gibberillic acid, a hormone that stimulates cell elongation.

An interesting side effect is that although growth is regulated, energy is still produced by photosynthesis. It has been shown that there can be substantial increases in stored energy when plant growth is uninhibited (Cooper et al.,1985). In contrast, researchers at the University of Illinois have investigated carbohydrate accumulation following standard application rates of plant growth regulators. Their results indicate that little-if any increase occurs, and furthermore, following release from regulation, a "rebound effect" significantly depletes carbohydrate levels.

Typically, plant growth regulators are applied when turfgrasses are actively producing shoot growth. What if we applied them at ultra-low rates during acclimation when energy is being stored? Would the plant accumulate more energy? Would this energy make the plant more freezing tolerant? Fruit tree research has demonstrated this response. Freezing stress resistance was enhanced with growth regulator applications and proposed to be related to alterations in membrane composition, reduced growth during acclimation and enhanced energy accumulation (Coleman and Estabrook, 1992).

Plant growth regulators applied during acclimation may prevent premature deacclimation during the transitional period the following spring. Minimizing the growth response to fluctuating freeze-thaw conditions could be a strategy for freezing stress tolerance by regulating growth and thereby prohibiting premature cell hydration.

While no definitive information exists to support this strategy, ongoing research will provide some baselines. Our project will be quantifying energy storage during acclimation of cool-season turfgrasses, the influence of management practices on energy storage, and the potential enhancement of freezing stress tolerance in relation to the use of plant growth regulators.

Summary

It is vital, when considering freezing stress, to maintain a broad perspective on this complex phenomenon. Simply, the most fascinating and frustrating aspect of freezing stress and winter injury research is the endless number of potential interactive causes: the inherent genetic potential of the plant, physiological alterations, the influence of management factors, and variable environmental conditions that exist in any one winter. All these factors individually and collectively appear to influence turfgrass responses to freezing stress.

Research programs throughout the world are studying various aspects of freezing stress. Also, turfgrass researchers draw on work from other crops and growing systems to understand stress responses in turf. Each contribution enhances our understanding of the stress tolerance process at work.

The goals of this discussion were to provide a general outline of the physiology of freezing stress and an experimental management approach to enhancing tolerance. Still, technology provides only limited control over this type of stress. Each turfgrass manager is challenged to accumulate and evaluate all available information to maximize turf survival. Hopefully, the amount of useful information on this important--and still poorly understood--area will increase.

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Tips to Maximize Winter Hardiness of Cool-Season Grasses

Fertility

1. Maintain a balanced fertility program. There is no conclusive evidence demonstrating the value of excessive potassium applications.

2. A well-timed, late-fall nitrogen fertilizer application made after top growth has ceased maximizes stored energy reserves.

Water

1. Slightly drier fall seasons often result in more winter-hardy plant material in the humid northeast. However, if your plants are susceptible to dessication because of open winters, be sure they are well-watered.

2. Low, wet areas where excess water accumulates and ice can be produced often results in problems with low temperature pathogens such as snow mold or suffocation from ice. Be sure to have adequate drainage on your high profile areas.

Miscellaneous

1. The use of turfgrass covers has produced variable results. Still, if your area is prone to open winters with little snow, the use of covers can aid in preventing dessication.

2. On low-cut turf, such as putting greens, it is advisable to increase the mowing height towards the end of the season to increase photosynthetic area and thereby maximize stored energy reserves.

Terms to Know

Winter Injury - nonspecific term commonly associated with any turf injury occuring during the winter period including: freezing stress, chilling stress, dessication, suffocation from ice, traffic and low temperature pathogens.

Freezing Stress - stress resulting from ice formation in or between plant cells that can cause irreversible damage if cells dehydrate or membrane dysfunction occurs.

Stem Apex (a.k.a. Crown) - a collection of cells located at the base of the turfgrass plant (often at the soil line) capable of dividing and producing plant parts such as leaves and stems.

Plant Growth Regulators - chemical substances capable of altering the normal growth and development of plants through disruption of cell division or cell elongation.

Nonstructural Carbohydrates - carbohydrates produced through photosynthesis that serve as sources of energy to drive the production of structural components including cell walls, membranes, etc..

Cytoplasm - the living substances in a cell excluding the nucleus.

Semipermeable Membrane - a membrane permeable to water but differentially permeable to other substances.

Fructans - the primary form of stored energy (nonstructural carbohydrate) in cool-season grasses.

Transitional Period - the period in late winter/early spring when cool-season turfgrasses are least winter-hardy, potentially hydrated, and consequently most susceptible to freezing stress injury.

For futher field tips see page 19