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PATHOLOGY

Winter Injury Understanding the Effects and **Research** Efforts

by Dr. Frank S. Rossi, Cornell University

ver the years, golf course management has been impacted by the introduction of various technologies. Technology has enabled the golf course superintendent to maintain higher quality conditions than would be expected if the technology was not available. Does it then follow that technology gives us control?

The answer is different depending on who you ask. Certainly, mechanical and chemical technology have provided tools used to achieve superior putting surfaces. Still, one must wonder how much we can actually control. When it comes to the various aspects of winter injury on our northern golf turf, the last few winters provided the harsh reality of exactly how much we can control - precious little.

Recent devastating losses from winter injury have revitalized interest in this otherwise neglected area, as evidenced by articles in popular trade magazines, conference topics and



Winter hardiness is extremely dependent on the species of turf.

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university research programs over the last few years.

Minimizing turf loss in the winter requires an improved understanding of the processes at work. Simply put, you can not protect what you 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." The irony of the situation is that estimates from industry surveys indicate 35 to 75 percent of all energy inputs in turf management in northern areas are as preparation for, and recovery from, the effects of winter. Despite these efforts, substantial turf loss can, and does, occur. The winter of 1992-93 in the Midwest U.S. and 1993-94 in the Northeast U.S. are just two examples.

Extensive turf loss from "winter injury" holds substantial environmental and economic consequences to the functional and aesthetic quality of recreational turf areas. Turf loss from winter injury, evident in the spring, results in increased weed encroachment, greater soil erosion and requires energy intensive re-establishment procedures to restore the environmental benefits of a contiguous and healthy sward.

What is needed is research: to answer the basic questions concerning the environmental and physiological conditions which result in freezing stress injury in cool-season turfgrasses. Understanding these conditions will aid in the development of winter-hardy plant material and allow for more energyefficient, environmentally sound management systems, which are less reliant on pesticides.

That there is a lack of information specific to turf management research is evident. In a recent literature review of lowtemperature stress in turfgrass, 85 percent of the literature cited represented cereal grain research. This type of research must be cautiously extrapolated to turfgrass systems. Annual crop programs can avoid winter stress periods through annual planting and harvesting practices, while perennial turf must suffer injury, enter dormancy or otherwise survive low-temperature stress.

Mechanisms of Injury

Ice Encasement: Turfgrasses respire energy throughout the winter. This physiological process requires gas exchange. Therefore, when winter conditions result in ice formation on the turf surface, the necessary gas exchange cannot occur and the area beneath the ice becomes anaerobic (lacking oxygen). In addition to the trapped gas from the turf, there is a substantial amount of gas given off from the soil since some microbes, such as the snow mold organisms, remain active in cold weather. This combines to create an environment that is hostile to turfgrasses.

Cool-season grasses have varying abilities to tolerate the conditions of ice encasement. For example, under research conditions, annual bluegrass can survive up to 60 days under ice, Kentucky bluegrass 100 days and creeping bentgrass 150 days. This turf loss is probably consistent with what most turf managers have experienced with the periodic damage to annual bluegrass under winter ice conditions.

Severe incidents of ice encasement are sporadic, occurring one out of every five years in most northern regions. But, management of these conditions, when it does occur, can be difficult.

The key to alleviating the problem is simply to break the ice to allow for adequate gas exchange. This can be accomplished by physically disrupting the ice. Some turfgrass managers have utilized core cultivation equipment fitted with solid, "hammer-like" tines to break the ice. Others apply a "blackening agent," such as dark compost or natural organic fertilizers (e.g. Milorganite), to the ice surface. On bright days, the compost absorbs heat, melts the ice and creates pores in the ice that allow for gas exchange.

Turfgrass Freezing Stress: Unfortunately, ice encasement is not the only challenge to turf from winter injury. Turfgrasses can be injured or killed during winter in the northern climates as a result of the singular or interactive effects of ice encasement, freezing stress, traffic, desiccation, soil frost-heaving and low-temperature fungi. 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.

During the transitional period between late winter and early spring, when freezing and thawing can occur, the plants can alternately experience warm, saturated conditions followed by rapidly freezing temperatures. These conditions can lead to freezing stress, where ice forms within the plant, causing severe cell dehydration.

Turfgrass injury from freezing stress is directly related to how, where and whether or not ice forms in cells of the turfgrass stem apex (a.k.a. crown); the primary 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 die. 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 tem-

peratures generally decline between 1° to 2°C per hour, thus allowing the cell time to adapt. However, when the temperature falls rapidly following warm or wet periods, freezing stress damage within the cell is possible.

The more common scenario for ice formation is between the plant cells or

intracellularly (Figure 1). As the ice crystal forms, it draws water molecules from inside the cell to expand the size of the crystal. As water is drawn from the cell, the cell becomes dehydrated. Dehydration causes a number of problems for the cell, not the least of which is membrane dysfunction, which allows even more water to flow out. Dehydration causes the degradation of other cellular components resulting in death of the cell. If enough cells in the crown are killed, the grass will not recover.

Freezing stress is thought to be the major factor affecting the survival of turfgrasses in the northern U.S.



Figure 1. As ice crystals form between cells, they draw water molecules from inside the cell to expand the size of the crystal. As water is drawn from the cell, the cell becomes dehydrated and can die. If enough crown cells are killed by ice crystals, the plant will die.

Freezing Stress Resistance

Plants naturally utilize various mechanisms to minimize intracellular ice crystal formation by holding water inside the cell tighter than the ice crystal can draw it out. These mechanisms of freezing stress resistance lie at the heart of strategies for survival.

Palta and Simon (1993) defined freezing stress resistance in plants as the plant's ability to achieve its genetic potential for growth, development and productivity by surviving freezing temperatures. They proposed avoiding extra- or intracellular ice formation and tolerating extracellular ice formation as the two primary survival mechanisms.

Avoidance: An interesting mechanism of avoidance called deep supercooling has been demonstrated with insects, mammals and some woody species. Deep supercooling occurs when the concentration of the soluble material in a liquid is raised to the point where temperatures below 32°F are needed for ice formation.

It seems reasonable that intracellular sugar accumulation during cold acclimation could, to some extent, lower the freezing point and avoid injury by allowing the cells to supercool. However, several researchers have observed only small (<7°F) reductions in freezing point. Supercooling is not viewed as the primary mechanism of freeze stress avoidance.

Tolerance: In 1980, a researcher stated that extracellular ice formation which results in cell plasmolysis and subsequent reduction of cell volume past a critical value is the principle cause, 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) would move from less to more concentrated solution. When the compartments reached equilibrium, net flow of water would cease. Plant cells with high solute levels in the cytoplasm, differentially permeable membranes and relatively rigid cell walls would permit net water movement to the interior, away from ice crystals forming extracellularly. Also in 1980, it was demonstrated that alteration of membrane function by incipient freezing injury could occur without changes in water permeability. Therefore, this would allow for a pressure which could resist plasmolysis and thus aid in maintaining membrane integrity under freezing stress.

Several turfgrass researchers have demonstrated a correlation between crown moisture content and turfgrass freezing stress resistance. However, the results were presented in a manner that made it difficult for the confirming researchers to detect small but important differences in freezing resistance. Clearly, however, an important tolerance mechanism is the reduced crown moisture levels that coincide with acclimation.

Cold Acclimation

Cold acclimation, or a plant's capacity to cold acclimate (enter dormancy), and later to deacclimate (break dormancy), has long been considered a significant factor determining freezing resistance. It has been suggested that some turfgrasses begin to cold acclimate during summer months and reach peak acclimation during mid-winter.

As winter progresses, several physiological alterations occur during incipient freeze-thaw cycles (characteristic of latewinter/early-spring conditions), such as nonstructural carbohydrate status, hormone levels (ABA, GA) and crown moisture content. These alterations can be corrected with plant growth. So, plants in late winter are physiologically in need of the benefits of growth.

However, it has become apparent over the last several years that the transitional period between winter and spring, often characterized by fluctuating freezing and thawing events, is the most crucial time for the occurrence of plant death as a result of freezing stress. During this time when plant energy reserves are low, the plant will respond to warming temperatures by stimulating or increasing growth.

When growth is stimulated, several physiological changes occur. The most sig-

nificant effect is the hydration of the plant tissues by water. As the crown hydrates to grow, it becomes more susceptible to freezing than it would be in a hardened state, since more free water is available.

Typically, we associate these situations with low, poorly drained areas, but tissues hydrate after the plant begins to grow from increased soil temperatures. This association with low areas may occur as a result of the standing water which is warmed by solar radiation. Once the water warms, heat is transferred to the soil, growth is stimulated, and the water is taken up. However, crown hydration is not confined to low areas; it will occur anywhere growth is stimulated and water is available for uptake.

Researchers have speculated for years that one of the single most important aspects for enhancing winter hardiness is delayed deacclimation or breaking of dormancy. This is most difficult with annual bluegrass, which is likely to break dormancy rapidly in the spring. In fact, researchers at the Prairie Turfgrass Research Center have quantified reduced hardiness of annual bluegrass following only eight hours of temperatures above 40°F. It was concluded that freezing tolerance was reduced 5° to 10°F following this slight warming.

It is important to understand a few of these basic principles, because they assist with determining the most effective management program for ensuring survival. Still, winter hardiness is extremely dependent on the species of turf growing.

Creeping bentgrass is one of the most winter-hardy species, while annual bluegrass is one of the more susceptible. Perennial ryegrass and tall fescue can be marginally hardy in the northern climates in the first few years following establishment. Mature stands can be more winter-hardy, especially if the soils are well drained and the area is somewhat protected.

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 would focus on several physiological areas, 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 can provide information for management strategies to minimize injury.

Energy Storage: Turfgrasses are not

entirely dormant during the winter. The plants continue to respire or deplete their energy supply as they overwinter, similar to how human physiology, especially breathing, is altered when we sleep. Therefore, entering winter with high levels of stored energy could provide several protective strategies.

The warming temperatures during the late winter/early spring transitional

period are thought to stimulate growth. This stimulation of growth sets the grasses up for the winter injury. Because not all types of turfgrasses deacclimate (or greenup) under the same temperature regimes, it seems reasonable that they would deacclimate because energy storage is below some critical level and there is a need to produce energy for survival, rather than be primarily temperature controlled. Further, it seems that elevated energy storage levels during the fall hardening-off process might make the plant less likely to deacclimate in the spring because energy storage would be above the critical level. Research at the UW-Madison was pointed at quantifying the critical level for several cool-season turfgrasses, specifically annual bluegrass.

Energy Storage and Cellular Water: As mentioned previously, ice crystal formation between the cells exerts a draw on the water inside the cell, resulting in cell dehydration. Plants that exhibit good cold tolerance appear to reduce cellular water levels during acclimation process. Still, when temperatures warm during the transitional period (winter to spring), cells hydrate.

As the ice crystal forms outside the cells, the area of formation has a lower concentration of water than inside the cell and water moves out of the cell to equalize the water concentrations. Late season maximiz-

Warming temperatures in late winter/early spring are thought to stimulate growth. This stimulation of growth sets the grasses up for winter injury. ing of solutes in the cell, like energy sources such as sugars and fructans, could reduce the concentration of water in the cell. This reduced concentration would prevent the water from passing through the membrane for ice crystal enlargement and the cell would stay hydrated and survive.

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 resulted in greater freezing stress tolerance. If the cereal grasses are not bred with the ability to store high levels of energy, the stress level will be high because cereal production strategies to maximize energy (late fertilization) are not practical. However, turfgrass management provides several potential strategies to enhance energy storage.

As with the cereal grasses, turfgrass managers can start with plant material that has demonstrated good freezing stress tolerance. However, because infestations of relatively unresistant annual bluegrass easily invades highly managed turf stands, using resistant varieties as the sole means of reducing winter injury makes this approach difficult at best.

It is possible, through primary cultural practices (mowing, fertilization and irrigation), to maximize energy storage during cold acclimation periods. Several researchers have investigated the role of potassium (K) with freezing stress tolerance. Since the role of K in plant energy production and storage remains unclear and information for testing has been conflicting and often inconclusive, just what role K plays in enhancing the cells' ability to retain water is unclear.

Factors that Influence Plant Winter Hardiness

Drainage: One of the most critical influences on winter injury, whether it is ice encasement, cell freezing, crown hydration (cell dehydration) or snow molds, is free-standing water available for freezing or to enhance disease activity. (Excessively wet fall periods prior to

winter will also reduce winter hardiness.) The importance of proper surface drainage cannot be stressed enough, especially on turf areas such as athletic fields and golf greens that are subject to high traffic in the late winter/early spring.

Fertility: For the grasses to maximize photosynthetic activity as stored carbohydrates, adequate, well-balanced nutrition must be available. Many studies have shown increased energy (carbohydrate) storage following late-fall fertilization.

Fertilizer products that have a high percentage of water-soluble nitrogen are ideal for this purpose. However, on sandy soils, care should be taken to use more moisturedependent, slow-release materials such as IBDU to ensure water quality.

The late-fall fertilizer treatment is best applied after top growth has ceased, which typically coincides with 7 to 10 days of a mean daily average temperature of 50°F or when nighttime temperatures fall below 30°F. This will ensure that any warming periods, which might stimulate top growth (Indian summer) and reduce hardiness, have passed. Depending on where you are in the north, this usually translates into late October. The carbohydrates developed from fertilizing prior to this temperature range are usually used up or may be used to increase leaf length going into winter.

Many turf managers apply excessive amounts of potassium (K) in the late season to enhance winter hardiness. Keep in mind, there is no conclusive evidence that indicates K levels above that which is required for adequate growth (indicated by soil test) will enhance winter hardiness. Furthermore, there may be severe consequences from excessive application of high salt content fertilizer, as suggested by researchers investigating bentgrass decline in the southeastern U.S.

Mowing Height: If we accept that grass leaves are where the energy is produced that enhances hardiness, it is then essential to have as much leaf surface area as possible available late in the growing season. Excessive close mowing, at or below the acceptable range for a particular species, will compromise energy production and reduce winter hardiness. It is advisable to raise the mowing height on putting greens if golfers will tolerate reduced ball roll distances.

Thatch: Excessive thatch accumulation will reduce winter survival. Thatch is less buffered from extreme temperatures, and plant crowns and other perennial structures which are elevated above the soil/thatch interface will be affected. In addition, thatch levels above one inch can promote desiccation and turfgrass disease incidence. Late season core cultivation to incorporate the soil from the cores into the thatch layer can assist with thatch decomposition and can also improve drainage by breaking through layers which can lead to increased hardiness.

Disease Management: Two research projects from Japan suggested that low temperature pathogens "sense" weak plants that may be more susceptible to infection. Subsequently, as previously indicated, maximizing plant health through proper acclimation with water management, fertility and mowing height could result in reduced snow mold activity. Nevertheless, species such as perennial ryegrass, creeping bentgrass and annual bluegrass are highly susceptible to disease and will still require preventative management to ensure survival.

Topdressing: Many turfgrass managers have used heavy, late season topdressing that serves to insulate the turf and protect the crown from desiccation in open or snowless winters. However, golf turf managers in the north-central U.S. have experienced problems with lateseason sand topdressing that might be dragged or brushed in. Researchers at the University of Wisconsin-River Falls have started a study investigating this management practice. Although results from the first year were inconclusive, it may be wise to avoid topdressing with highly angular/sharp sand and then brushing it in. This practice can abrade the leaf surface and may accelerate desiccation.

Traffic: Of all the management factors that are under the control of the turfgrass professional, minimizing traffic during periods when the soil is frozen or just when turf is not actively growing can be the most difficult. Players want to use the turf and that conflicts with what is known regarding maintaining healthy plants. While there is limited data on early season play, estimates suggest that active

play during the "shoulders" of the growing season can subsequently require many weeks of active growth for recovery. Therefore, if possible, minimize traffic when the plants are dormant or the soil is frozen.

Turf Covers: The use of synthetic, protective turfgrass covers for enhancing winter survival, has provided variable results over the years. Recent studies from Laval University in Quebec have indicated that snow is the best insulator and should be kept on as long as possible. The next best thing is

any cover that uses an air layer to insulate the turf from extreme temperature and moisture. Keep in mind that covers accelerate green-up in the spring and can result in reduced winter hardiness if temperatures drop suddenly.

Plant Growth Regulators

Plant growth regulators (PGRs) were introduced more than 40 years ago for application to utility turf to reduce their mowing requirements by inhibiting turfgrass shoot growth. Today, plant growth regulators are used to improve turf color, reduce clippings, suppress seedheads and improve green speed.

A field study of fall-applied PGRs on cereal hardiness resulted in an increase in the average survival of winter cereals. These effects, however, were not consistent from year to year, indicating the complexity of the problem. Winter cereals, especially the less hardy genotypes, are known to have reduced freezing stress tolerance from January to March, even though they are constantly exposed to subzero temperatures. It is possible that the regulation of the acclimation and deacclimation process through the use of PGRs may involve a component

The importance of proper surface drainage cannot be stressed enough, especially on turf areas such as athletic fields and golf greens that are subject to high traffic in the late winter/early spring. of a complicated stress response. Still, the interaction of freezing stress and PGRs might provide insight to solving the previously uncontrollable problem.

Certain classes of PGRs increase cold hardiness or winter survival by reducing the production of gibberellic acid and could increase the photosynthate partitioning (storage) in the crown of the plant. Research in 1993 indicated that a post growth-inhibition period, six to eight weeks following a PGR application, resulted in a resurgence of growth and a concomitant decrease in total carbohydrate levels. This resurgence of growth would need to be minimized through the timing and rate of applications, in order to avoid any inappropriate growth activity.

Trinexapac-ethyl is a class A plant growth regulator labeled for use in turfgrass management for reducing shoot growth without causing significant injury. Trinexapac-ethyl inhibits the gibberellin biosynthesis process late in the pathway. This results in an increase in abscissic acid (ABA) levels that decrease shoot growth and increase carbohydrate storage, which may improve freezing stress tolerance.

Triazole plant growth regulators such as paclobutrazol are class B PGRs that act much earlier in the gibberellin biosynthetic pathway. It has been reported that ABA levels are increased in plants grown under triazole regulation. It has also been suggested that the combination of lowered gibberellic acid and increased ABA levels increase stress tolerance during chilling or freezing.

Theoretically, late fall applications of a plant growth regulator could improve the winter hardiness of plants by altering their carbohydrate status during cold acclimation when energy is being produced and used for storage, rather than for top growth. This treatment could coincide with the gradual cessation of shoot growth, the initiation of the hardening process, membrane alteration and accumulation of photosynthate. This could lead to a plant with enhanced cryoprotective features and an increased energy source, allowing it to withstand the incipient freeze-thaw periods. **Controlled Environment Studies:** Plant growth regulator effects on winter injury of annual bluegrass were studied in a growth room at the University of Wisconsin-Madison's Biotron.

The objectives of this project were: (1) to determine if commonly used plant growth regulators affect the winter hardiness and turf quality of annual bluegrass throughout the fall and spring;

(2) to determine the relative freezing tolerance of annual bluegrass during fall and winter acclimation while under growth regulation; and

(3) to determine if trinexapac-ethyl increases carbohydrate concentrations, thereby improving winter hardiness under controlled environment conditions.

Preliminary studies indicated that in general, lower rates of PGRs enhanced winter survival, while higher rates had a detrimental effect. It was also evident that wet conditions during acclimation made the plants more susceptible to injury. Subsequent experiments simulated fall and winter acclimation, and the late winter/early spring deacclimation process on plants maintained in relatively saturated soil.

For the experiment, 7-cm plugs of annual bluegrass were extracted from the same fairway where a field study was being conducted concurrently to ensure consistency in biotype population between the field and controlled environment studies.

The plants were then maintained in a greenhouse with 12-hour day length for a month, simulating summer conditions. The plants were hand-watered to prevent moisture stress and mowed with a clipper approximately every other day. Pots were then treated with trinexapac-ethyl and permitted to acclimate. Then temperatures were reduced two degrees per hour to 5°C day temperature and 2°C nighttime temperature. This daily regime was maintained for three weeks.

Secondary acclimation was attained by lowering the temperature of the room one degree per hour to 0°C, where it was maintained as both the daytime and nighttime temperature for three weeks. Secondary

acclimation conditions were then followed by a 48-hour warm up to 8°C daytime temperature and 5°C nighttime temperature, permitting deacclimation.

Finally, plants were removed from the Biotron after one and three weeks of primary hardening, one and three weeks of secondary hardening, and after the 48-hour deacclimation. A variety of freezing temperatures were then imposed to determine the tolerance of the plants untreated and treated with trinexapac. At the same time, plants were being harvested to determine carbohydrate content, to correlate with changes in freezing stress tolerance.

Results from the controlled environment experiments indicated that freezing stress tolerance could be enhanced with ultra-low rates of trinexapac. The amount of enhancement appeared to be slight and not well correlated with observed increases in carbohydrate content. Plants treated with trinexapac seemed to deacclimate more rapidly when exposed to warming temperatures than untreated plants. However, at the lowest rate, treated plants had a greater relative freezing tolerance than untreated plants.

The variability we observed with the carbohydrate concentration was consistent with results observed by previous researchers. Further experimentation under controlled environmental conditions will be needed to specifically quantify the physiological state of the plant prior to PGR application.

Field Studies: Field experiments to evaluate winter injury and spring greenup were conducted on a golf course fairway composed primarily of annual bluegrass. Plant growth regulator applications were made at various rates and times throughout the fall at Nakoma Country Club in Madison, WI, from 1994-96. (This particular area is a regular site of significant winter injury.)

Plots were rated for injury related to the application in the fall and subsequently for winter injury and recovery in the spring.

Significant injury occurred in each of the three years we conducted the study. In year one, applications made in September and October at standard rates caused significant turf injury, evident by November. Consequently, most plots were killed by the spring. In years two and three, we reduced the rates to 6%, 3% and 1.5% of the normal rates and observed less injury in the fall. However, the winters were harsh and resulted in a widespread kill that was attributed to

severe ice encasement. Interestingly, in year two, plots that survived the winterkill had been treated with low rates of PGRs and had produced significantly more tillers, which were more robust when compared to untreated plants. Nevertheless, in all three years, plots required over 8 weeks to recover to acceptable quality, a situation that would be completely unacceptable to

As a result of the lack of field efficacy, we are hesitant to make strong recommenda-

golf superintendents.

tions for this strategy under field conditions. Still, increased tillering evident in the spring on treated plots and results observed under controlled environment studies indicate that some benefits might be available using different application strategies, i.e., timing, rate and product.

Summary

It is vital, when considering freezing stress, to maintain a broad perspective on this complex process. Simply, the most fascinating and, at the same time, most frustrating aspect of freezing stress and winter injury research is the endless number of potential interactive causes: from the inherent genetic potential of the plant material, to alterations of physiology, to the influence of management factors and the variable weather conditions that exist in any one winter.

Research programs throughout the world are tackling various aspects of freezing stress. Also, turfgrass researchers can draw on work from other crops and growing systems for some guidance. Each contribution enhances the understanding of the processes at work.

Increased tillering evident in the spring on plots treated with growth regulators and results observed under controlled environment studies indicate that some benefits might be available using different timing, rates and products. The goals of this discussion were to provide a general outline of the physiology of freezing stress and a look at an experimental management approach to enhancing tolerance. However, as of this writing, technology still only provides limited control of this type of stress. In the final analysis, each golf course superintendent and turf manager is challenged to accumulate and evaluate the available information on turfgrass winter injury to maximize survival of the turf at their managed site. Hopefully, this has provided some useful information on this important, and still poorly understood, area.

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