A PRACTICAL RESEARCH DIGEST FOR TURF MANAGERS

TurfGrass TRENDS

Volume 6, Issue 2

February 1997

IN THIS ISSUE

Physiology of Turfgrass Freezing Stress Injury

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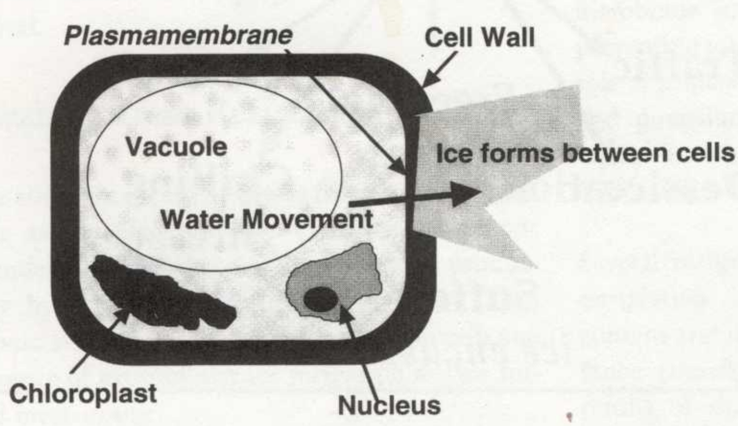
Through the years, golf course management has been impacted by the introduction of various technologies. Technological advances have enabled golf course superintendents to maintain higher quality turf and playing conditions than could be expected if technology was unavailable. Does it follow then that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

Ice Formation & Plant Cells



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TurfGrass TRENDS

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TurfGrass TRENDS is published monthly. ISSN 1076-7207. The annual subscription price is $180.00.

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

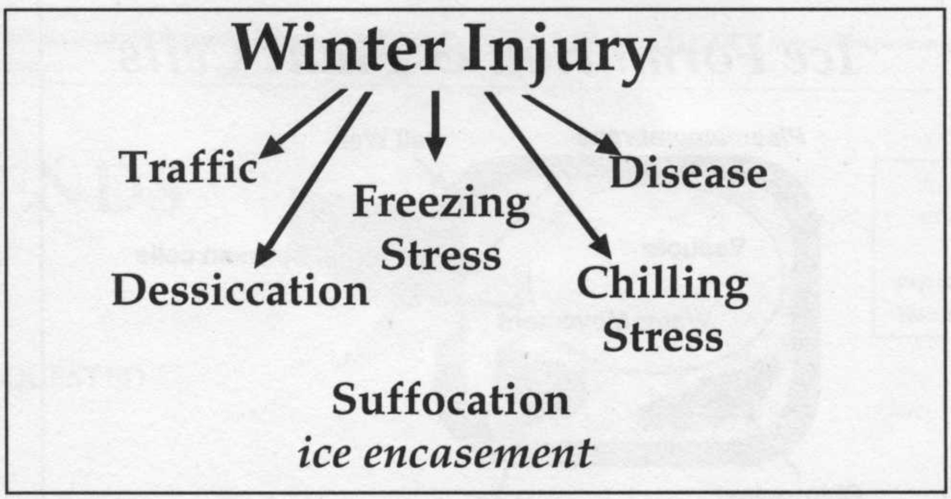

Figure 1

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.

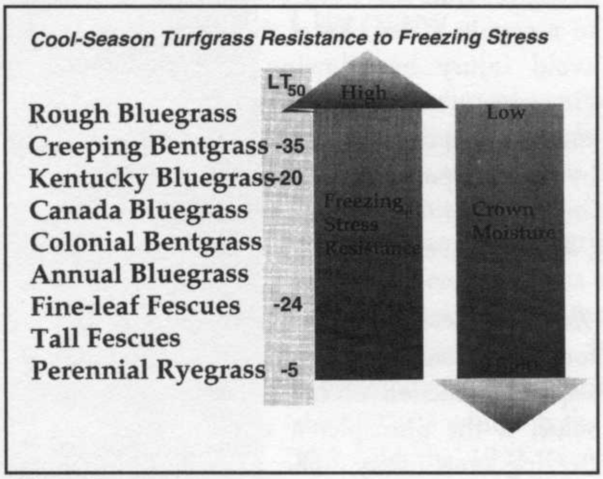

Figure 2

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.

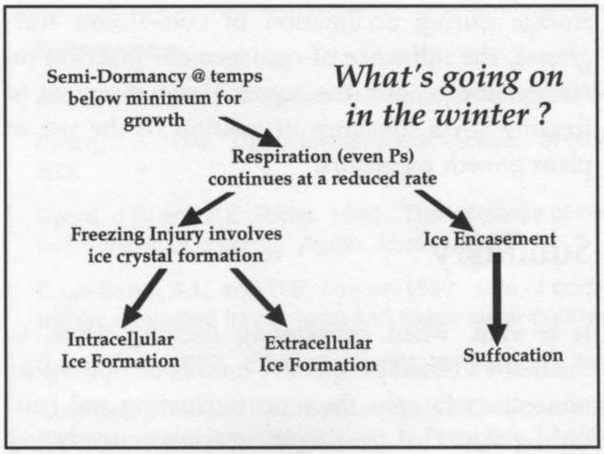

Figure 3

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

Advanced Concepts in Turfgrass Nutrition

by R. E. Schmidt and Xunzhong Zhang
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Justus von Liebig, a mid-nineteenth century chemist known as the "Father of Agricultural Chemistry," postulated that exhausted soil was simply the result of mineral nutrient removal. Based on his philosophy, the giant mineral fertilizer industry was developed. Because of von Liebig's hypothesis, it is frequently assumed that specific levels of mineral nutrients in plant tissues reflect plant performance as well as nutrient availability in the soil. However, the mineral content of individual plants of the same cultivar which exhibit different growth responses is relatively small (Driessche and Wareing, 1966). Also, the rate at which grass leaves grow in response to fertilization is poorly correlated with turfgrass quality (Mehall et al., 1984). It has been observed that vigorously growing turfgrass managed under a high nitrogen regime is often more susceptible to environmental stress than slow growing turfgrass (Green and Beard, 1969; Funk et al., 1967).

Various environmental stress factors influence the onset and rate of senescence (aging) in turfgrasses. Senescence is enhanced by pest attack, high temperature, low light intensity, low or high soil moisture, anaerobic soil conditions, nutrient excess, nutrient deficiency, and toxicity of pesticides (Couch, 1995).

Clipping production of cool-season turfgrasses is generally greatest in spring, followed by a depression during mid-summer (Landschoot and Waddington, 1987; Woolhouse, 1981). Hull (1992) demonstrated that this seasonal pattern is not eliminated by elevated N fertilization. He speculated suppression or inhibition of summer growth in cool-season grasses also may be a response to hormonal change. This seems reasonable, as all plant growth aspects are influenced by hormones.

Mineral Fertilization Impact on Growth and Organic Substances in Turfgrasses

Since early 1960, turfgrass researchers have been studying the relationship between mineral nutrition and the concentration of plant organic substances. Their objective has been to understand the relationship between effective fertilization and seasonal changes. It has been documented that, during the growing season, elevated nitrogen fertilizer levels have a negative impact on root growth of cool season grasses (Adams et al., 1974; Goss and Law, 1967; Madison, 1962). Under such conditions, carbohydrates in storage organs are used to stimulate vegetative shoot growth and thereby decrease the partitioning of energy available for root production (Miflin, 1980). During winter, when shoot growth and respiration are depressed because of low temperatures, the products of photosynthesis are shifted from the leaves to sites of non-structural carbohydrate storage and enhancement of root development. Powell et al. (1967) reported that N applied to turf during late fall initially inhibited root growth, but subsequent root development was stimulated. Apparently this was due to increased photosynthate accumulation associated with early spring green-up, resulting from elevated chlorophyll synthesis promoted by winter nitrogen availability.

Environments favoring rapid growth often reduce the non-structural carbohydrate content of cool-season grasses (Hull, 1992). Watschke et al. (1972), in a study involving ten Kentucky bluegrass cultivars, showed that those cultivars high in non-structural carbohydrates after four weeks at elevated temperatures were also high in apparent photosynthesis and foliar yield. Turfgrass tolerance to heat stress in July has been related to carbohydrate reserves by others (Wehner et al., 1985; Schmidt and Blaser, 1967). It has also been observed that elevation of nitrogen fertilization reduces heat tolerance of cool season turfgrass due to low levels of carbohydrate reserves in the leaves of vigorously growing plants (Watschke et al., 1972; Pellet and Roberts, 1963; Wehner and

Watschke, 1981). Exposing turfgrasses to low night temperatures improved growth of plants subjected to high day temperatures (Schmidt and Blaser, 1967; Watschke et al, 1970). This was also correlated with an elevated carbohydrate content in the grass.

Wehner and Watschke (1984) suggested that net protein synthesis in cool-season turfgrasses is a heat sensitive process. Heat stress may result in the denaturation of proteins, alteration of membrane fluidity and permeability, and the unfolding of nucleic acid. Heat tolerance of photosynthesis is enhanced by an increase in the number of double bonds (unsaturation) in membrane lipids (Gombos et al., 1994).

Phytohormones Impact Turfgrass Growth

Investigations concerning the control of plant growth by organic chemicals commenced prior to the turn of the century. It was proposed that specific substances controlled cell enlargement and cell division. Charles and Frances Darwin in 1880, demonstrated that light detected by the tip of a grass coleoptile (shoot of a dark-grown seedling) transmits a signal by some means to the lower part, causing the coleoptile to bend. This influence later was identified as organic compounds synthesized in one part of the plant and translocated to another. These compounds, now called hormones, cause physiological responses at very low concentrations. The concept that hormones significantly impact plant growth was initiated in this country during the mid-1930's (Skoog, 1994). A prevalent skepticism concerning the existence of plant-growth-promoting hormones persisted and was exhibited at the time when Fritz Went, in 1935, was unable to demonstrate the Avena coleoptile curvature tests for auxins in Pittsburgh, which at that time was severely air polluted. The negative attitude Dr. Went met prompted him to investigate the effect of air pollution on plant hormonal responses. This led to the Haagen-Smit identification of major toxic compounds in smog for which a National Medal of Science was awarded. Following this work with auxin, Dr. Folke Skoog, in an arena of much skepticism, was able with co-workers to demonstrate the influence of cytokinin hormones on plant growth stimulation. Although skepticism still prevailed, studies of the biological activity of hormones continued. In the 1950's, it was shown that auxins and cytokinins interact in regulating growth and development of roots, shoots, and flowers. Gibberellins and cytokinins promote leaf formation. This knowledge currently is used by scientists working to develop transgenic plants.

Frankenberger and Arshad (1995) have compiled a comprehensive review of the literature that provides evidence to support the idea that exogenous sources of hormones can influence plant growth and that the physiological effects of these materials cannot be replicated by an equivalent application of mineral nutrients. Many of the compounds capable of regulating plant growth and development have been identified. These are referred to as plant growth regulators (PGRs). When produced endogenously, they are called phytohormones. Both terms relate to the hormones which include auxins, gibberellins, cytokinins, ethylene and abscisic acid (Nagri, 1995).

Although plants are capable of synthesizing phytohormones in response to environmental changes, phytohormone production is enhanced in response to applications of certain materials. Exogenously-applied humic acid, triazole compounds, as well as cytokinin materials can enhance endogenous phytohormone synthesis.

Turfgrass research at Virginia Tech during the last ten years has shown that exogenous applications of cytokinin materials increased the endogenous cytokinin concentrations of grasses (Yan, 1993). Applications of cytokinin materials to turfgrasses have delayed senescence and enhanced tolerance to drought, salinity and nematode stresses. Applications of seaweed, an excellent source of cytokinin material, in combination with auxins applications, have been associated with enhanced root development of various turfgrasses grown under stress environments (**Figure 1**).

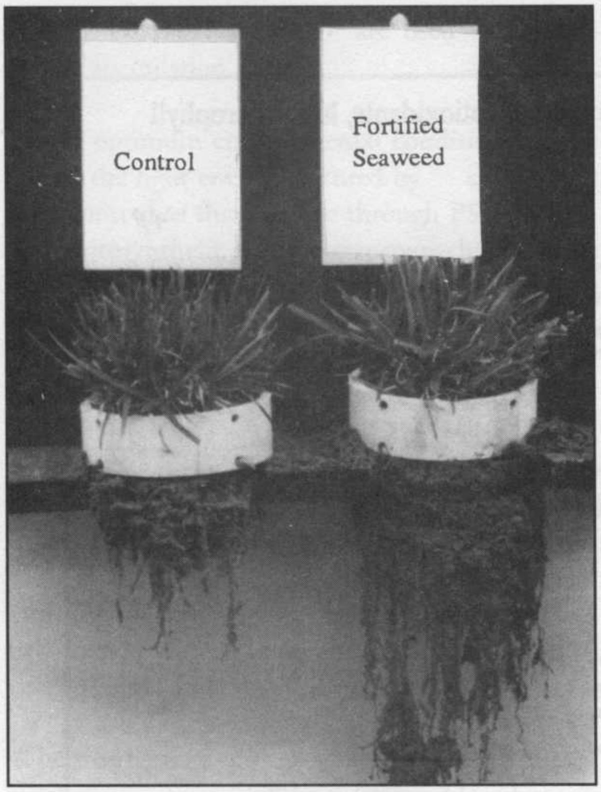

Figure 1. Early spring (1996) root enhancement of September 1995 established tall fescue when treated with fortified seaweed in November 1995.

The influence of phytohormones on the physiological and biochemical mechanisms of plants is complex. Ongoing research has provided some information for explaining the mechanisms involved. Plants may acclimate to stressful environments by changing their membrane composition and fluidity (Hale and Orcutt, 1987). Yan (1993) showed the application of cytokinin-like materials to ryegrass increased membrane fluidity and salt stress tolerance. His results indicate that cytokinins may serve as a signal for membrane modification in plants under stress.

Phytohormones Influence Endogenous Antioxidants

Various environmental stresses (drought, heat, salt, etc.) disturb normal metabolism of plants and result in oxidative stress, characterized by excess accumulation of toxic oxygen free radicals (Larson, 1988; Zhang and Kirklan, 1994). These activated oxygen species, which result when excess electrons generated from photosynthesis or respiration are captured by elemental oxygen (O_2), may damage lipid, protein, nucleic acid and other macromolecules. Although plants possess antioxidant defense systems to remove these free radicals, the antioxidant levels may not be high enough to reduce the activated oxygen levels developed under severe stress. Stimulation of antioxidant activity may condition plants to survive oxidative buildup and improve stress tolerance and growth. Cytokinins can scavenge free radicals directly or prevent the formation of free radicals, as well as serving as a signal for triggering the activity of other antioxidant systems. Mozafar (1994) has reviewed research that shows nitrogen fertilizer may increase, decrease or have no effect on antioxidant content in plants. It appears antioxidants tend to increase with nitrogen supply to a maximum, then decrease with further nitrogen increases. Therefore, N concentration of turfgrass may be considered excessive when additional N fertility causes antioxidant content to decline. This may be complicated, as indicated by numerous reports that show plants supplied with nitrogen from an NH_4^+ source of fertilizer contained less ascorbic acid than when supplied with a NO_3^- source. Also, it has been suggested that excess N fertilization may reduce the allocation of available carbohydrates for antioxidant synthesis. This may explain why actively growing turfgrasses stimulated by high N fertilization are more susceptible to certain environmental stresses.

Phytohormones Impact Chlorophyll Fluorescence

One of the major targets for injury caused by environmental stress is the photosynthetic apparatus. Absorbed light energy drives photosynthetic electron transport through photosystem II (PSII) and photosystem I (PSI), leading to the oxidation of water (oxygen evolution), reduction of $NADP^+$ to NADPH, and photophosphorylation generating

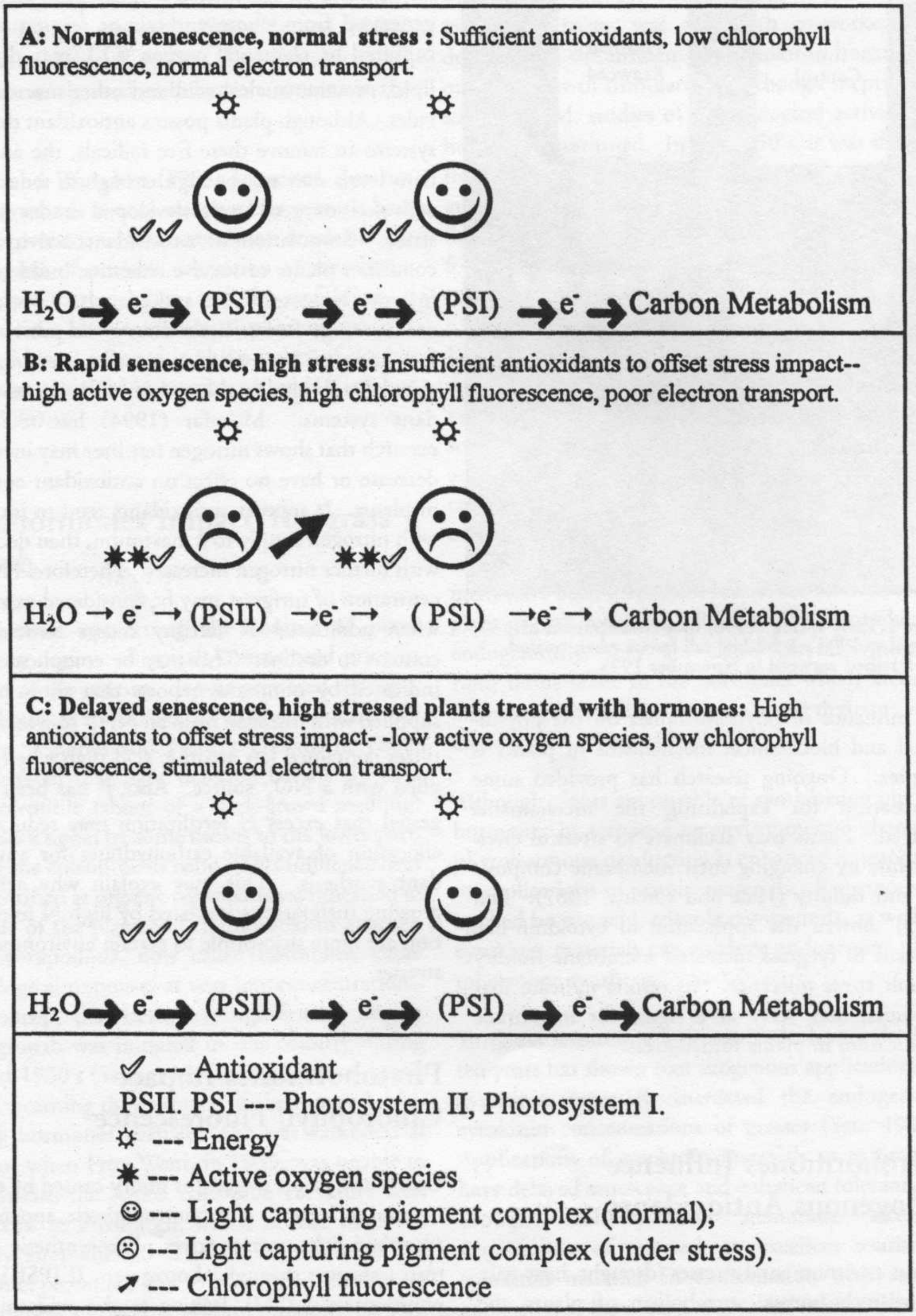

Figure 2 **Photosynthetic apparatus as influenced by stress and antioxidant status.**

ATP. NADPH and ATP are used for carbon (CO^2) assimilation.

Under optimum environmental conditions, about 3% of the light energy captured by chlorophyll is re-emitted as fluorescence through PSII. When the photosynthetic electron transport chain accepts and transfers electrons, chlorophyll fluorescence remains low. When electron carriers are reduced, they cannot accept and transport electrons and the transference of light energy is impaired and chlorophyll fluorescence increases rapidly (Miles, 1990). Any alteration of the photosynthetic apparatus can affect the level of fluorescence. Therefore, the health or capacity of a plant's photosynthetic apparatus or the level of oxidative damage (insufficient antioxidants) can be measured and monitored in a non-destructive way by in vivo chlorophyll fluorescence (**Figure 2**).

When turfgrass is grown under stress, the photosynthetic apparatus will be suppressed by photoinhibition or active oxygen damage. The level of fluorescence then will increase rapidly. Excess N reduces stress tolerance by suppressing antioxidant activity, including cytokinin content. This negative impact of N can be revealed by elevated chlorophyll fluorescence. It is possible to monitor antioxidant status, state of mineral nutrition, and functioning of photosynthetic apparatus by tracing chlorophyll fluorescence kinetics.

Increasing Antioxidants and Lowering Chlorophyll Fluorescence of Turfgrasses

In turfgrass systems, the abiotic or biotic environments are frequently suboptimal; consequently, the grass will experience some sort of stress. Since plant development is influenced by internal as well as external environments, it is possible to influence internal (endogenous) metabolism with the application of external (exogenous) materials.

Research at Virginia Tech has shown that the concentration of antioxidants (alpha-tocopherol, ascorbic acid, 8-carotene, superoxide dismutase) in turfgrasses increased significantly in response to exogenous applications of hormones, particularly auxins and cytokinins.

Applications of seaweed extracts, which contain high levels of cytokinins and auxins (Crouch, 1995), and humic acids, which are rich in auxin activity (Hamence, 1944; O'Donnell, 1973), enhanced antioxidant levels of turfgrasses under both favorable moisture and drought stress conditions thereby stabilizing the synthetic capabilities of the plant. We have observed that treating tall fescue with seaweed extract increased the SOD (antioxidant) activity and lowered chlorophyll flourescence throughout the growing season.

When the ratio of the variable chlorophyll flourescence (FV) to the maximum chlorophyll flourescence (FM) at 690 nm wavelength is calculated, an estimate of photochemical efficiency (FVM=FV/FM) of PSII can be ascertained. Photochemical efficiency may be referred to as chlorophyll activity. From a current field study using urea as the nitrogen source, we demonstrated that non-PGR-treated bentgrass grown under low fertility had higher chlorophyll activity than when grown under high fertility (**Figure 3**). This corresponded with an increase in concentration of the antioxidant SOD within the leaves. However, under both fertilizer regimes, chlorophyll activity increased when seaweed extract was applied. The antioxidant content of bentgrass grown under low fertility increased only slightly after seaweed extract treatment. On the other hand, the grass grown under the high fertility regime had a three-fold antioxidant (SOD) increase following seaweed extract applications. These results indicate that the antioxidant demand of heavily fertilized bentgrass can be met with exogenous applications of materials containing growth regulating substances.

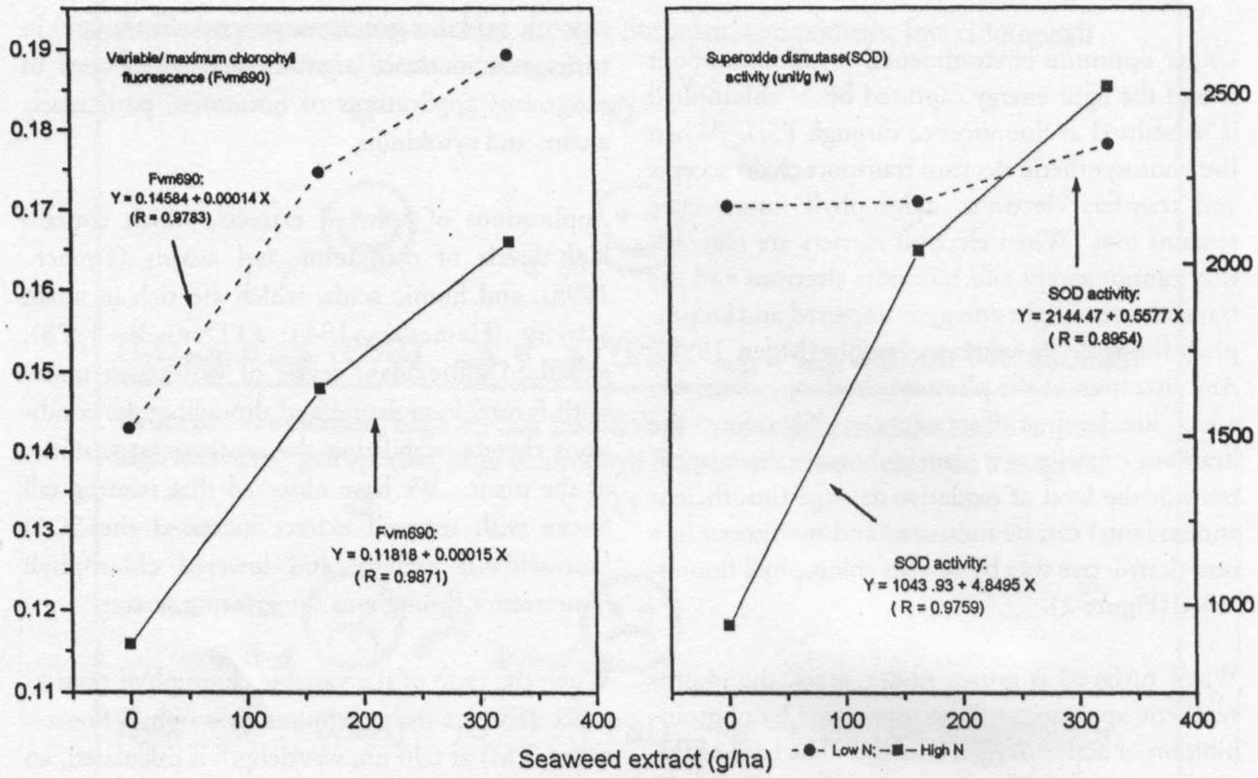

Figure 3 Chlorophyll fluorescence and endogenous antioxidant SOD activity as influenced by Seaweed extract and N level in creeping bentgrass (TRC, 1996)

Application

Current studies are investigating practical means for increasing the efficiency of the photosynthetic apparatus in turfgrasses when exposed to stress. Research has shown that materials such as seaweed extracts and humic acid applied as part of a turfgrass nutritional program stimulate the endogenous production of phytohormones. This, in turn, reduces chlorophyll fluorescence and increases endogenous antioxidants, resulting in greater turf tolerance to environmental stresses. Results obtained show that the use of materials that enhance plant production of phytohormones increases both chlorophyll activity and endogenous antioxidants, which can be used to improve turfgrass growth and development under stress conditions.

Infection and disease development are associated with turfgrass subjected to stress conditions (Nelson, 1996). The greater the level of stress, the greater the fungicide efficacy required to achieve adequate levels of disease control. Therefore, using materials that reduce the impact of environmental stress is a valuable component of integrated pest management (IPM) systems used in the production of turfgrasses. Incorporation of this knowledge into turfgrass systems will enable the turfgrass manager to assess plant health to a much greater extent than currently possible with soil and plant tissue analysis. The turfgrass manager will be able to bring the nutritional status of turf into an advanced dimension.

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Xunghong Zhang has B.S. and M.S. degrees from the Agricultural University of Hebei, People's Republic of China, where he is a professor in the Crop Science Department. He came to Virginia Tech in 1992 as a visiting scholar and now is pursuing his Ph.D. in turfgrass physiology. His current research activities involve antioxidant response of and stress tolerance in turfgrasses.

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Terms to Know

Chlorophyll Fluorescence - light energy surplus to that utilized in photosynthesis (PSII) is dissipated as fluorescence. Fluorescence excited by 690 nm laser diode and passed through a 690 nm filter may be detected by a photodiode.

Endogenous - referring to a natural substance produced within the cells or an organism.

Exogenous - referring to a substance entering the cells of an organism from the outside. Exogenous substances may be natural or synthetic but always are introduced from the external environment of an organism.

Free Radical - a highly reactive chemical containing an unpaired electron. Free radicals are often strong oxidizing agents and are destructive of biological molecules such as enzymes, nucleic acids and membranes.

Hormone - a naturally occurring organic chemical produced in one organ and translocated to another organ where it modifies growth or function. In plants, such chemicals are referred to as phytohormones.

Non-Structural Carbohydrates - carbohydrates involved primarily in energy storage or transport, including soluble sugars, starch and fructans. Structural carbohydrates are those incorporated into the structure of cell walls, including cellulose, hemicellulose and pectins.

Photosynthate - carbon compounds produced directly by photosynthesis. As generally used, the term refers to simple sugars that are transported within green leaf cells and among tissues and organs of a plant.

SOD - Superoxide Dismutase is an enzyme that metabolizes the highly reactive free radical of oxygen, superoxide. It is considered an antioxidant because it eliminates superoxide, a strong oxidizing agent that can damage organic molecular structures.

Unsaturated Lipids - lipid or fat molecules composed of long-chain fatty acids which contain from one to three double bonds between carbon atoms. Such unsaturated lipids are more oxidized and have a lower freezing temperature than saturated lipids. Unsaturated lipids built into the lipid core of membranes make the membrane more fluid during low temperatures and times of stress.

Frost, Firewood, Spike-Free Footprints and Winter Putting Conditions

by Mike Huck
USGA Agronomist

While most northern courses have closed down, southern courses are growing in their winter overseeding. Everyone soon will be hearing those famous winter comments: "What do you mean we can't tee off until 9:30 am?"; "Can't you just turn the sprinklers on and melt the frost?"; and "But the thermometer at the bank down the street said 36 degrees--that's not freezing!" Yes, it's the frost season again.

It is sad but true that many golfers do not understand the importance of allowing greens to thaw before play begins and, even worse, do not appreciate the long-term consequences caused by placing concentrated traffic on turf that is not actively growing. Since turfgrass leaves are composed primarily of water, the entire plant (internally and externally) can rapidly freeze when temperatures drop. Comparing a grass plant's leaf to a paper straw filled with water provides a simple and graphic illustration of this point. While an unfrozen straw would flex under pressure, a frozen straw would snap in two, damaging the tissue.

The frost is a great time to encourage cutting some firewood to warm your greens. When the sun is positioned low on the horizon, long shadows cast by trees shading eastern and southern exposures have significant impact on how early greens thaw and become playable. Golfers and club officials are probably more likely to approve tree removal in order to avoid delaying starting times than for any other reason imaginable. Reducing early morning shade not only melts frost and gets players on the course earlier, but also helps maintain warmer soil temperatures improving winter and spring growth. Additionally, scientific evidence shows that early morning sunlight on greens is extremely important to grow healthy turf and deep roots. Compared with greens growing in cleared areas, those in shaded surroundings are much slower to respond in spring and often enter summer in a weakened state.

It does not matter whether you are in Denver, Phoenix or Los Angeles, traffic damage during the winter months when growth and recovery are limited can be devastating. The thinning and compaction caused by concentrated spike traffic during cold weather can require many weeks to heal when conditions again become favorable for growth. Consequently, a great number of courses that I visit in the Denver and Salt Lake City areas have banned metal spikes, at least through the cold season. This is done because of the obvious improvement in turf quality spike alternatives offer, and once players witness firsthand the improvement in putting quality through the winter, the program is often continued year round. The results have been so dramatic at neighboring Denver courses that municipal facilities, like those operated by the City of Aurora, have gone spike-free, too! In fact, the spike-free movement is so strong in Colorado that the Colorado Golf Association requires contestants to wear spike alternatives in their events throughout the year.

So, if your golfers are interested in getting on the course earlier in the morning and improving winter and spring playing conditions, I have two suggestions: cut some firewood to warm the greens and make your footprints spike-free.

Mike Huck *is an agronomist in the United States Golf Association's Western Region office. He provides turf advisory services for Colorado, Nevada, Arizona, Utah and parts of California. Mike attended California State Polytechnic University where he earned a degree in Ornamental Horticulture with a specialty in turfgrass.*

Field Tips: Winter Turf Injury in Coastal New England

by Larry Pakkala, CGCS
Woodway Country Club

The winters of 1993 and 1995 in the northeastern United States were severe to say the least. Turf managers at the finest golfing facilities struggled to repair damaged turf (mostly Poa annua on putting greens) to open their courses for the season. To compound the problems, we had unseasonably cold temperatures until the end of May, making seed germination next to impossible. Superintendents tried all kinds of tricks and trades to facilitate turf recovery before summer's onslaught. It was almost comical.

The ice that had formed beneath the snow was a condition many of us had never faced. When the ice melted, the effects of crown hydration were not at first apparent. But seven days later--WHAMMO! Large areas of Poa annua had died from crown hydration on greens once covered by ice.

What We Learned

If at all possible, do not let surface ice form on greens. There is some thought that snow removal is not necessary; however, in my experience it is extremely beneficial. The key is not the snow cover itself but ice that forms underneath as a result of rain following snow. Ice forms because water from rain and melting snow cannot drain through the already frozen surface. Our practice is to remove by squeegee any surface water that puddles before it freezes--a labor intensive practice, perhaps, but better than having to repair damage resulting from crown hydration. The USGA Green Section strongly recommends installing subsurface drains to low areas of greens where water collects. Additionally, installing a short piece of pipe to the surface with a cover speeds water drainage during the winter. When the threat of freezing has passed, simply replace the surface pipe with soil and sod and install a metal plate 4 inches under the sod to allow easy location the following winter.

__Larry Pakkala__, CGCS, has been the property manager at Woodway Country Club in Darien, Connecticut, for 15 years. He received his degree from Pennsylvania State University. He has served as president of the New York Metropolitan GCSA and the Tri-State Turf Research Foundation (New York, New Jersey and Connecticut).

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- Bermudagrass and Fertilizers
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