Winterkill can have a devastating effect on spring course conditions.

Exploring the Use of Plant Growth Regulators to Reduce Winter Injury on Annual Bluegrass (*Poa annua* L.)

Increasing energy reserves will reduce winterkill and enhance plant health.

by FRANK S. ROSSI, Ph.D., and EMILY J. BUELOW

EACH YEAR, golf turf managers in the northern United States are faced with the possibility of widespread turf loss as a result of winter injury. Winter injury, or winterkill, as defined in Beard (1973) is a nonspecific term commonly used to represent any injury that occurs to turf during the winter period. Stresses leading to injury that could be included in this discussion are a result of desiccation, low-temperature pathogens, traffic, chilling, suffocation from ice encasement, and freezing stress. Furthermore, because winters vary from year to year, each stress could be imposed individually or collectively and result in turf loss. The myriad of stress and growing environments leaves a golf course superintendent wondering how to maximize turf health before winter or how to recover dead turf the following spring.

Freezing Stress

DiPaola and Beard (1992) summarized the forms of winter injury that can occur in cool-season turfgrass systems due to mechanical and dehydration stresses. These stresses are related to the movement of water out of the cell during slow cooling (1-2°C per hour) of the plant tissue as ice forms between the cells. Ice formation within plant cells is thought to be rare because of the historically slow rate of temperature drop and the ability of the cell to supercool, i.e. dehydrate and concentrate the cell sap so that the freezing point is lowered.

Ice develops in the larger vessels between the plant cells because water between the cells has a lower solute concentration than the water within the cells. Ice formation in plant tissue usually occurs by the time temperatures drop to between -1 to -3°C. Living cells in the plant are surrounded by the plasma membrane, which prevents the growth of ice crystals in the cell. Therefore, ice formation is confined to the space between the cells and is absent within the cell.

Extracellular freezing cannot directly produce freezing injury since there is no direct contact between the ice and the interior of the cell. Water moves from the inside to the outside of the cell to support the growing ice crystal, resulting in cellular dehydration. Physiologically, cellular water lost to the growth of extracellular ice can result in severe dehydration and death as a result of mechanical stress due to the collapse of the cell walls.

The primary area of concern for survival of turfgrasses is the meristematic region, or the crown of the plant. Freezing stress injury is directly related to how and where ice forms in the cells of the turfgrass crown. Within the crown tissue itself, there are different levels of cold hardiness. The lower part of the crown, the region that initiates root development, is less hardy than the upper part of the crown. This is especially true in annual bluegrass (Beard and Olien, 1963). Therefore, in the spring, it is possible for plants to appear healthy, only to become necrotic in the absence of functional roots associated with lower crown damage.

Many changes occur in the plant cell during the induction of freezing, such as changes in membrane lipids, proteins, and non-structural carbohydrates. Davis and Gilbert (1970) demonstrated that starch and total non-structural carbohydrate content of turfgrasses increase during fall hardening, and showed a strong association between these reserves and freezing stress tolerance. However, the contribution of each of these changes toward increased freezing tolerance is not well understood.

Carbohydrates in the cells are thought to interfere with ice crystal growth, thereby reducing mechanical injury to the cell during freezing. For example, trehalose, a disaccharide, functionally replaces free water bound to membranes and proteins, and thus confers structural stability during desiccation. It could be hypothesized that if carbohydrate levels were increased through alternate management practices, freezing stress tolerance could be enhanced as a result of many cryoprotective mechanisms operating concurrently.

Plant Growth Regulators?

Plant growth regulators (PGRs) were introduced more than 40 years ago for application to utility turfs to reduce mowing requirements by inhibiting turfgrass shoot growth. With improved technology and the introduction of newer materials, the effectiveness and potential utility in turfgrass management have increased. 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 resulted in an increase in the average survival of winter cereals (Gusta et al., 1988). These effects, however, were not consistent from year to year, suggesting the problem is a complex one. 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 sub-zero temperatures. It is possible that the regulation of the acclimation and deacclimation process through the use of PGRs may involve a component 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 (GA) and/or could increase the photosynthate partitioning in the crown of the plant (Hanson and Branham, 1987). Spak et al. (1993) indicated that a post growth-inhibition period six to eight weeks following a PGR application results in a resurgence of growth and a concomitant decrease in total carbohydrate levels. This resurgence of growth would need to be minimized by the timing and rate of applications.

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 inhibition 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. ABA levels are thought to increase in plants grown under triazole regulation, and it has been suggested that the lowered gibberellic acid and increased ABA levels increase stress tolerance during chilling or freezing (Gusta et al., 1996).

Theoretically, late fall applications of a plant growth regulator could improve

A cold room at the University of Wisconsin - Madison Biotron, where cold hardiness is studied.

the winter hardiness of plants by altering their carbohydrate status during 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 application could lead to a plant with enhanced cryoprotective features and an increased energy source, allowing it to withstand the incipient freeze-thaw periods.

The objectives of our research project are: (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 and thereby improves winter hardiness under controlled environment conditions.

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

Seven-centimeter plugs of annual bluegrass were extracted from the same fairway where a field study was being conducted to ensure consistency in biotype population between the field and controlled environment studies. The plants were then maintained in a greenhouse with a 12-hour day length for a month, simulating summer conditions. The plants were hand watered to prevent moisture stress and mowed with clippers approximately every other day.

Pots were then treated with trinexapac-ethyl and permitted to acclimate. Temperatures were reduced two degrees per hour to 5°C day temperature and 2°C nighttime temperature. This 5°/2°C daily regime was maintained for three weeks.

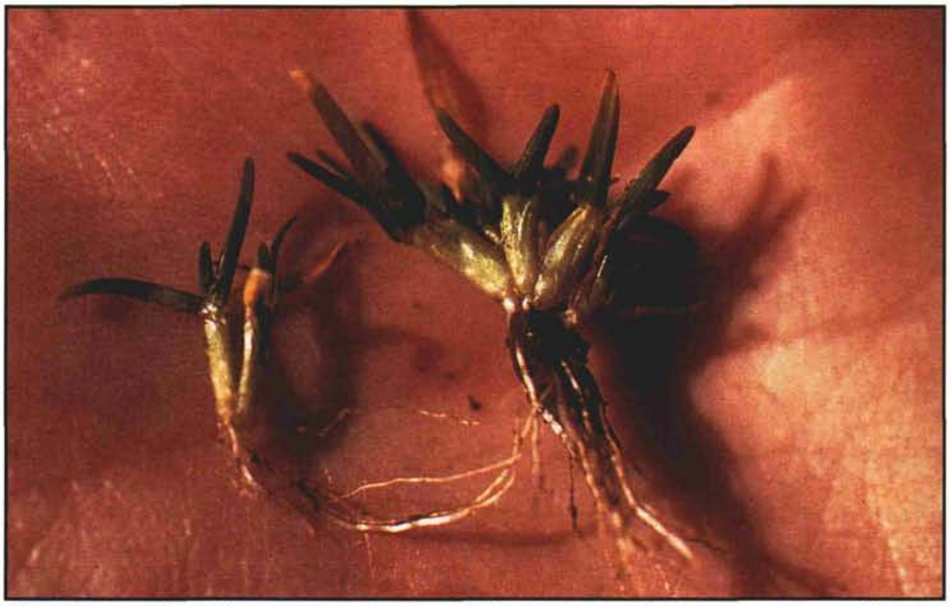
Secondary acclimation was attained by lowering the temperature of the room one degree per hour to 0°C, where it was maintained as both the daytime and nighttime temperature for three weeks. Secondary acclimation conditions were then followed by a 48-hour warm-up to 8°C daytime temperature and 5°C nighttime temperature, permitting deacclimation.

Freezing tolerance of plants was estimated by an experimental technique that utilizes a circulating glycol bath. Specifically, plants were removed from the Biotron after 1 and 3 weeks of primary hardening, 1 and 3 weeks of secondary hardening, and after the 48-hour deacclimation. A variety of freezing temperatures were imposed to determine the tolerance of the plants untreated and treated with trinexapac. Simultaneously, plants were being harvested to determine carbohydrate content to correlate with changes in freezing stress tolerance.

Results from the controlled environment experiments indicate that freezing stress tolerance can be enhanced with ultra-low rates of trinexapac. The amount of enhancement initially appears to be slight and not well correlated with observed increases in carbohydrate content. Plants treated with trinexapac appear to deacclimate more rapidly when exposed to warming temperatures than untreated plants. However, at the lowest rate, the 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 Eagles and Williams (1992). Further experimentation will be needed in controlled environments to specifically quantify the physiological state of the plant prior to PGR application.

Injury observed in November on plots that were treated with PGRs one and two months earlier. Note checkerboard yellowing. Uninjured plots were untreated.



The plant on the right was treated with an ultra-low rate of trinexapac the previous fall and exhibited increased tillering the following spring as compared to the untreated plant.

Field Studies

Field experiments to evaluate winter injury and spring green-up 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 (Randy Smith, golf course superintendent) in Madison, Wisconsin, from 1994 to 1996. 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 use rates caused significant injury, which was evident in November. Consequently, most plots were killed by the spring. In years two and three, the rates were reduced to 6%, 3%, and 1.5% of the use rates, and we 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 were treated with low rates of PGRs and had produced significantly more tillers that were more robust as compared to untreated plants. Nevertheless, in all three years, plots required more than eight weeks to reach acceptable quality, a situation that would be completely unacceptable to golf course superintendents.

As a result of the lack of field efficacy, we are hesitant to make strong recommendations 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 and rate.

Conclusions

The logic that motivated this research effort remains sound, i.e. enhancing plant health through increased energy reserves will reduce winter kill. However, as with all exploratory research, we have raised more questions than we have answered. The search for enhanced stress tolerance, be it temperature, moisture, or traffic will be tedious as we realize that there are no singular answers to complex interactive responses. Until researchers find improved methods of quantifying plant health other than visual quality or biomass production, we will be limited in our ability to enhance stress tolerance.

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