

## PROJECT OVERVIEW

Low temperature injury and winterkill are major limitations in the management of annual bluegrass on putting greens and fairways in New England. Low temperature acclimation, or hardening, is an important aspect of plant survival of freezing temperatures; however, turfgrasses can undergo deacclimation during freeze-thaw events throughout late winter and early spring, thus leaving the plant susceptible to freezing injury. The loss of cold hardiness in response to deacclimating temperatures may be one important factor associated with greater injury to annual bluegrass compared to creeping bentgrass. Based on our studies thus far, we have determined that freezing injury of some annual bluegrass ecotypes was associated with more rapid deacclimation in response to warming events. We also identified some important differences in carbohydrate, hormone, and protein metabolism traits between annual bluegrass and creeping bentgrass that can account for their differences in freezing sensitivity associated with deacclimation.

### Research Studies

Premature deacclimation associated with warming periods during winter and early spring can negatively impact turfgrass freezing tolerance and lead to winterkill. Field observations suggest that annual bluegrass and creeping bentgrass differ in their capacity to resist deacclimation, which can contribute to differences in winter injury potential among these species. Therefore, research is necessary to understand the factors that trigger deacclimation in grasses and to identify plant traits that contribute to enhanced deacclimation resistance and freezing tolerance. The specific objectives of our research were to first determine how sensitive annual bluegrass and creeping bentgrass were to brief warming events, and then to examine early physiological changes associated with deacclimation sensitivity of these two species, including carbohydrate, hormone, protein metabolism parameters.

In our initial studies, we evaluated deacclimation sensitivity for one annual bluegrass ecotype (previously shown to exhibit freezing sensitivity) compared to one creeping bentgrass cultivar ('L-93'). The grasses were exposed to progressively lower temperatures until reaching approximately 28°F in controlled environment chambers, which helped to induce cold acclimation. In order to determine what would happen if a brief warming spell occurred, the grasses were then exposed to deacclimation treatments that consisted of warming the chambers until the soil temperatures reached 39°F, 46°F, or 54°F for up to 5 days at each temperature. In all the experiments, we found that annual bluegrass never achieved the same level of freezing tolerance as creeping bentgrass in response to cold acclimation, determined as the lethal temperature at which 50% of plants were killed (LT<sub>50</sub>). In response to warming temperatures, we found that both grass species lost some level of freezing tolerance. What was surprising was that even one day of exposure to 39°F caused both grasses to lose some freezing tolerance compared to grasses that had been maintained at around 28°F. As expected, this response was exacerbated the longer the plants were exposed to the warmer temperatures (for example, higher deacclimation was observed at 46°F and 54°F compared to 39°F). Overall, the annual bluegrass ecotype demonstrated a greater loss in freezing tolerance when exposed to deacclimation compared to creeping bentgrass (Figure 1). It is important to note, however, that at higher temperatures, creeping bentgrass was also sensitive to deacclimation and losses in freezing tolerance. In order to better understand the physiological basis for deacclimation in grasses, we monitored changes in carbohydrate metabolism in response to exposure to simulated mid-winter warming events. We found that annual bluegrass leaves were greener and had higher water content compared to creeping bentgrass when exposed to deacclimating temperatures (Figure 2). As a result, photosynthesis and respiration rates increased very rapidly during deacclimation, suggesting that metabolic and physiological activities of annual bluegrass were activated earlier in response to warmer temperatures compared to creeping bentgrass (Figure 3). Based on the examination of

different types of carbohydrates in the grass leaves and crowns, we found that creeping bentgrass specifically accumulated higher amounts of important storage carbohydrates, called fructans, during cold acclimation. These types of carbohydrates have been shown to be critical in the overwintering capacity of many cool-season grass species, including forage grasses. In response to deacclimation, the fructan content decreased for both species, but was still significantly higher for creeping bentgrass (Figure 4). The ability to retain higher levels of these fructans may contribute to better overall freezing tolerance creeping bentgrass, particularly when warming periods are quickly followed by freezing temperatures. Interestingly, when comparing a freezing tolerant annual bluegrass ecotype to a freezing sensitive ecotype, we also found that the more tolerant ecotype maintained higher levels of fructans during deacclimation. These results support our previous observations that annual bluegrass can exhibit a more rapid capacity to restore carbohydrate metabolism compared to creeping bentgrass in response to warming events. Although this may provide a competitive advantage for spring green-up, the greater uptake of water and the breakdown of important cryoprotective compounds, such as fructans, likely predispose annual bluegrass to freezing injury response to mid-winter warming events. Differences in the genetics of annual bluegrass ecotypes growing on greens, fairways, and tees may also influence how quickly these metabolites are used up, and account for variation in winter injury potential of annual bluegrass across the same or different golf courses. Lastly, our most recent studies have also helped us to identify specific proteins that were only detected in creeping bentgrass, as well as differences in the signaling of plant hormones, such as auxins and abscisic acid, which are now further being investigated in the lab.

We thank the NERTF and the USGA for the generous funding of these projects, which helped to support the graduate studies of two students, including one Ph.D. student, Lindsey Hoffman, and one M.S. student, Xian Guan, at the University of Massachusetts. Lindsey is currently a post-doctoral scientist in the turf and switchgrass breeding program at Rutgers University, and Xian has gone on to pursue her Ph.D. at Cornell University.

Figure 1. Increased injury to annual bluegrass (top row) compared to creeping bentgrass (bottom row) when plants were exposed to 46°F for 1 day, followed by freezing temperatures

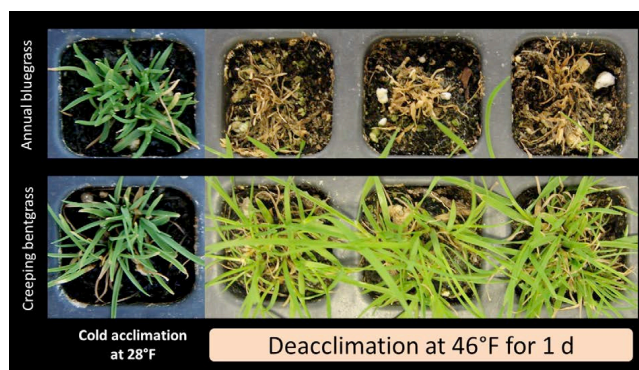


Figure 2. Differences in the color and leaf water content of annual bluegrass and creeping bentgrass in response to deacclimation at 46°F for one day.

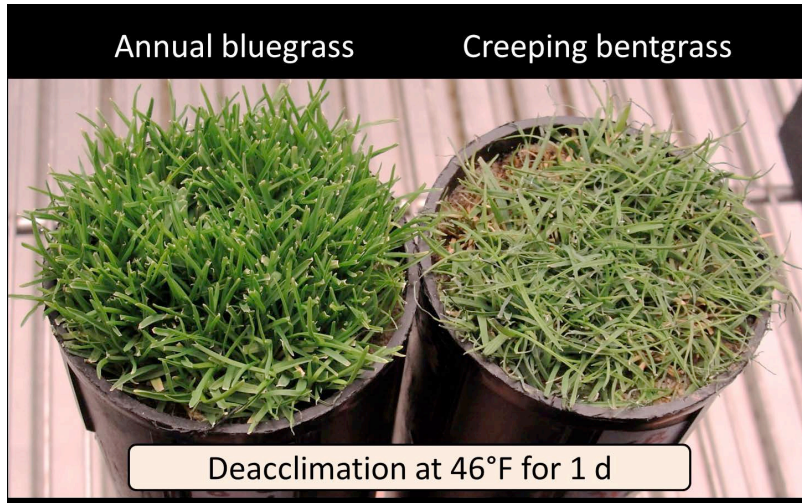


Figure 3. Changes in plant photosynthetic rates of annual bluegrass and creeping bentgrass plants were moved from cold acclimation conditions at 28°F to deacclimation conditions at 46°F for up to 5 days. Annual bluegrass was observed to have active photosynthesis even within 1 day of the warming event compared to creeping bentgrass.

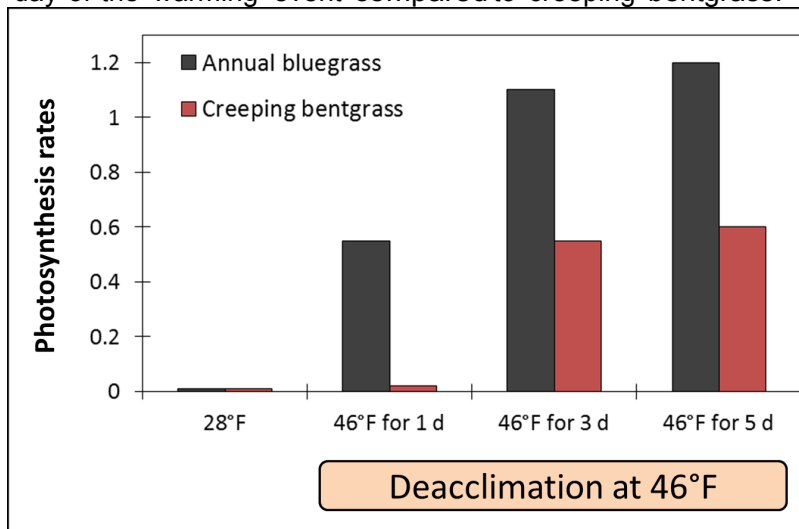


Figure 4. Changes in the amounts of the important storage carbohydrates, fructans, in the crowns of creeping bentgrass and two annual bluegrass ecotypes (one generally freezing tolerant and one freezing sensitive). Although the fructan content of all three grasses decreased in response to deacclimation at 46°F, creeping bentgrass maintained significantly higher amounts of this storage carbohydrate throughout deacclimation. In addition, the freezing tolerant annual bluegrass ecotype also had higher crown fructan content compared to the freezing sensitive ecotype.

