Effects of Ethylene Regulation on Creeping bentgrass and Annual bluegrass Survival of Ice Cover

Kevin Laskowski and Emily Merewitz

Introduction

Ice damage to annual bluegrass (*Poa annua;* ABG) and creeping bentgrass (*Agrostis stolonifera;* CBG) golf course putting greens is a significant problem in many parts of the world and climate change may increase ice incidence. ABG and CBG are both susceptible to ice cover, with ABG being more susceptible (killed at approximately 45 to 70 d) and CBG being more tolerant (killed after 100+ days) (Tompkins et al ; 2004). The primary cause of death to turfgrass under ice sheets is most likely from oxygen depletion and toxic gas accumulation (Pessarakli, 2008). ABG was shown to have toxic gas accumulation and metabolic activity resulting in toxic gases (Aamlid, et al., 2009). CBG and ABG are known to be differential in production of the gaseous hormone ethylene; ABG produces a lot of ethylene but is not sensitive to the hormone, whereas CBG produces less with greater sensitivity (Fioriani et al., 2002). Ethylene improves plant tolerance of freezing stress by increasing antifreeze protein expression in winter rye plants (Yu et al., 2001) but reduces freeze tolerance in other species (Shi et al., 2012). Ethephon application (effective ethylene) was deleterious to warm season turfgrasses for winter survival (Munshaw et al., 2010). To our knowledge, whether ethylene may be associated with the difference in tolerance to various winterkill stresses, particularly ice stress, of cool-season turfgrasses has yet to be investigated. Since ABG is less tolerant of ice stress, we hypothesize that high levels of ethylene production may have negative effects on ice stress survival and treatments that inhibit ethylene production may improve survival under ice stress. This study aimed to investigate the effects of ethylene regulation on ABG and CBG survival of ice cover.

Discussion

Ethephon is a commonly used chemical in turfgrass management for annual bluegrass flowering and seed head control. Turfgrass managers that may be using ethephon for control of annual bluegrass flowering, particularly as a replacement for mefluidide, may see a decrease in turf quality depending on environmental conditions and concentration of ethephon applied. The novel finding here is that ethephon treatments could significantly reduce spring recovery following winter conditions either under no ice or ice-covered conditions at the rates used in our study. Through antioxidant and lipid peroxidation analysis we found that ethephon treated annual bluegrass had decreased antioxidant activity and increased lipid peroxidation when compare to the untreated control in both leaf and crown tissues. This decrease in antioxidant activity may be correlated with a decrease in annual bluegrass recovery after -4^{o}C treatment. Suggesting that lower antioxidant activity and increased lipid peroxidation may result in decreased annual bluegrass recovery.

One indication of turfgrass acclimation is the changes in cell membrane fatty acid content. During acclimation, plants that are cold tolerant may increase concentrations of unsaturated fatty acids while reducing saturated fatty acids in their cell membranes (Shang et al., 2006). Ethylene inhibitory treatments generally increased unsaturated fatty acids and decreased saturated fatty

acid concentrations when compared to the untreated controls. Specific types of change in individual FAs may also play a role in cold tolerance. Retain treated annual bluegrass had greater linolenic concentrations when compared to the untreated control after 80 day at -4^{o}C. Linolenic acid has been found to be involved in lipid based signaling involving jasmonic acid in response to insect and pathogen attack (Farmer et al., 1992). This shows that changes in plant cell membrane fatty acid composition may alter other plant defense mechanisms which could help a plant survive winter conditions. Research observing cold tolerance in plants shows that increases in linolenic acid also increase cold tolerance of plants during early growth stages (Iba, 2002). Ethephon is an effective ethylene treatment. Fatty acid analysis of annual bluegrass treated with ethephon resulted in ethephon having greater saturated fatty acid content when compared to the untreated control. This could be an indication that effective ethylene applications may decrease the ability of annual bluegrass to acclimate to winter conditions decreasing the ability of recovery during the spring.

Ethylene inhibition treatments influenced turfgrass recovery from winter conditions and were found to influence FA composition of annual bluegrass tissues. Our results indicate that Retain increased annual bluegrass recovery after low temperature treatment at -4^{o}C after 40 and 80 days of treatment when compared to an untreated control. Ethylene inhibition products Retain and AVG were observed to increase unsaturated fatty acid concentrations and decreasing saturated fatty acids. Retain is commercially available in the produce industry for reducing or inhibiting ethylene production in fruits (Clayton et al., 2000). Retain, has been tested previously on creeping bentgrass in Arkansas to determine the maximum concentration prior to phytotoxicity (Strunk et al., 2010). Their research showed that applying a concentration of 91.5 g A^{-1} Retain or less did not decrease turf quality. Our research also supports this in that Retain did not decrease turf quality when compared to the untreated control. However, to date, no research has evaluated ethylene production from annual bluegrass or creeping bentgrass under ethylene inhibition treatment. Our research showed that Retain did not have a measurable effect on ethylene production in the field when compared to the untreated control.

One plant defense against ice formation is the accumulation of antifreeze proteins. Antifreeze proteins accumulate in the apoplast making them available to bind to the surface of ice formation changing the rate at which ice may accumulate in plant tissue (Griffith et al., 1992) Retain and AVG treated annual bluegrass were observed to have greater antifreeze protein in leaf tissue when compared to the untreated control. This is contrary to what researchers have found in winter rye leaves where ethylene inhibitory treatments had lower apoplastic protein concentrations (Yu et al., 2001).

These results indicate that ethephon treatment as an effective ethylene application of annual bluegrass may be detrimental to spring recovery by increasing lipid peroxidation, increasing saturated fatty acid content, and decreasing antioxidant activity when compared to untreated annual bluegrass after winter conditions. Products that are ethylene inhibitors could potentially be beneficial to annual bluegrass survival during the winter through increases in antioxidant activity and increases in plant cell membrane unsaturated fatty acid contents when compared to untreated annual bluegrass. Thus, turfgrass managers in northern climates need to

be cautious of recommendations for annual bluegrass seedhead control and should use caution with using ethephon during the fall, since a reduction in annual bluegrass survival over winter may occur.

In conclusion, ethephon applications made weekly from the beginning of October through mid-November can be detrimental to annual bluegrass spring recovery depending on the severity of winter conditions. Ethephon was found to have decreased recovery after 0 and 20 days of -4^{o}C treatment. However, after 40 and 80 days of -4^{o}C treatment, it was no different from the untreated control. Measuring antioxidant activity of annual bluegrass after low temperature treatment at -4^{o}C under ice and no ice cover suggests that ethephon applications reduces activity of antioxidant enzymes and increases lipid peroxidation which may result in a decrease in regrowth during the spring. Ethylene inhibitory products, such as Retain, may serve as a means of increasing annual bluegrass spring recovery in low temperatures and ice cover persist for greater than 40 days during the winter. This suggests that effective ethylene applications alter annual bluegrass physiology reducing the percent of recovery occurring in the spring while ethylene inhibitor treatment may increase annual bluegrass recovery during extended periods of below freezing temperatures and ice cover.

References

Aamalid TS, Landschoot PJ, Huff DR. 2009. Tolerance to simulated ice encasement and Microdochium nivale in USA selections of greens-type Poa annua. Acta Agriculturae Scandinavica, 59: 170-178.

Bradford M.M. 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein dye binding. Anal Biochem 72:341-347.

Chance, B. and A.C. Maehly. 1955. Assay of CAT and POD. Methods Enzymol. 2:764–775.

Clayton, M., Biasi, W. V., Southwick, S. M., & Mitcham, E. J. (2000). ReTain™ Affects Maturity and Ripening of Bartlett' Pear. HortSci. 35:1294-1299.

Dhindsa, R.S., P.P. Dhindsa, and T.A. Thorpe. 1981. Leaf senescence: Correlation with increased levels of membrane permeability and lipid peroxidation, and decreased levels of SOD and CAT. J. Expt. Bot. 32:93–101.

Farmer, E. E., C.A. Ryan. 1992. Octadecanoid precursors of jasmonic acid activate the synthesis of wound-inducible proteinase inhibitors. The Plant Cell. 4:129-134.

Fiorani F, G. Bögemann, E.J. Visser, H. Lambers, and A. Laurentius, C.J. Voesenek. 2002. Ethylene emission and responsiveness to applied ethylene vary among Poa species that inherently differ in leaf elongation rates. Annu Rev Plant Physiol. 129:1382-90.

Giannopolitis, C.N. and S.K. Ries. 1977. SOD. I. Occurrence in higher plants. Plant Physiol. 59:309–314.

Griffith M., P. Ala, D.S.C. Yang, W.C. Hon, B.A. Moffatt. 1992. Antifreeze protein produced endogenously in winter rye leaves. Plant Physiol. 100:593-596.

Heath, R.L. and L. Packer. 1968. Photoperoxidation in isolated chloroplasts. Arch. Biochem. Biophys. 125:189–198.

Hon W.C., Griffith M, Chong P, Yang D.C.S. 1994. Extraction and isolation of antifreeze proteins from winter rye (Secale cereal L.) leaves. Plant Physiol. 104:971-980.

Jespersen D, Yu J, Huang B 2015. Metabolite responses to exogenous application of nitrogen, cytokinin, and ethylene inhibitors in relation to heat-induced senescence in creeping bentgrass. PLoS ONE 10(3): e0123744. doi:10.1371/journal.pone.0123744

Larkindale, Jane, and Bingru Huang. 2004. Thermotolerance and antioxidant systems in Agrostis stolonifera: involvement of salicylic acid, abscisic acid, calcium, hydrogen peroxide, and ethylene. Journal of plant physiology 161: 405-413.

Krans, J.V., and K. Morris. 2007. Determining a profile of protocols and standards used in the visual field assessment of turfgrasses: A survey of national turfgrass evaluation program-sponsored university scientists. Appl. Turfgrass Sci. doi:10.1094/ ATS-2007-1130-01-TT

Munshaw, G.C., E.H. Ervin, J.S. Beasley, C. Shang, X. Zhang, and D.J. Parrish. 2010. Effects of late-season ethephon applications on cold tolerance parameters of four bermudagrass cultivars. Crop Sci. 50:1022-1029.

Pessarakli M. 2008. Handbook of turfgrass management and physiology. Taylor and Francis, CRC Press, New York, NY.

Shang, C., Zhang, X., Munshaw, G., & Ervin, E. 2006. Determination of Fatty Acid Composition of Turfgrass by High- Performance Liquid Chromatography. Communications in soil science and plant analysis, 37(1-2), 53-61.

Shi, Y., Tian, S., Hou, L., Huang, X., Zhang, X., Guo, H., & Yang, S. 2012. Ethylene signaling negatively regulates freezing tolerance by repressing expression of CBF and type-A ARR genes in Arabidopsis. The Plant Cell, 24:2578-2595.

Strunk, D., D. Karcher, and M. Richardson. 2012A. Phytotoxicity of Aminoethoxyvinyl-glycine Hydrochloride on Creeping Bentgrass. Arkansas Turfgrass Report 2010, Ark. Ag. Exp. Stn. Res. Ser. 593:35-38.

Tompkins, D. K., J. B. Ross, and D. L. Moroz. 2004 Effects of ice cover on annual bluegrass and creeping bentgrass putting greens. Crop Sci. 44, 2175-2179.

Nakano, Y. and K. Assada. 1981. Hydrogen peroxide is scavenged by ascorbate specific POD in spinach chloroplasts. Plant Cell Physiol.22:867–880.

National Turfgrass Evaluation Program. 2015. A guide to NTEP turfgrass ratings. National Turfgrass Evaluation Program, Beltsville, MD. http://www.ntep.org/reports/ratings.htm#quality (verified 9 Dec. 2016).

Rylski I., L. Rappaport, and H.K. Pratt. 1974 Dual effects of ethylene on potato dormancy and sprout growth. Plan Physiol. 53(4): 658-62.

Yu X.M, M. Griffith , and S.B. Wiseman. 2001. Ethylene induces antifreeze activity in winter rye leaves Plant Physiology 126:1232-1240.