# ANSWERS TO SOME QUESTIONS GOLF COURSE SUPERINTENDENTS HAVE CONCERNING THE USE OF BIOSTIMULANT

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In talking with several golf course superintendents we became aware that some confusion might exist in determining the best way to incorporate biostimulant treatments to enhance a turfgrass nutrient program. The conversation with these fellows was reminiscent of a recent research article written by a good friend in which she claimed that research is like a good mystery story (Allen, 2000). She indicated that Sherlock Holmes would have made a good research agronomist, as he would thoroughly understand the hazards of a rush to judgment. In A Scandal in Bohemia he tells Dr. Watson "It is a capital mistake to theorize before one has data. Insensibly one begins to twist facts to theories, instead of the theories to suit facts" (Doyle, 1987). I feel that many turfgrass managers are much like Sherlock Holmes. A cultural practice becomes elementary only when the facts are realized.

Let us review some of the facts that have been realized by the data concerning biostimulants that we have collected over the past ten years. Turfgrass managers may be aware of our reports that a biostimulant is an organic material that, when applied in small quantities, enhances plant growth and development such that the response cannot be attributed to application of traditional plant nutrients. These materials also may be referred to as "positive plant growth regulators" or as Beard recently coined "metabolic enhancers".

Our research has documented that applications of materials fitting the above definition have conditioned turfgrasses to tolerate environmental stresses and improve grass growth, particularly root development. Applications of biostimulants have been shown to improve turfgrass photochemical activity (an estimate of photosynthetic efficiency) and overall quality when the turf is subjected to low soil moisture, dollar spot, non-target pesticide applications, nematode infestation, high soil salinity, high UV light intensity, and heat.

HOW MUCH AND HOW OFTEN SHOULD A BIOSTIMULANT BE APPLIED?

How may a turfgrass manager know how much of which material to use? How often should a biostimulant be applied? These are difficult questions to answer since biostimulants are manufactured from different materials and formulated at various concentrations. (Some that have low concentrations of biostimulating materials may seem less expensive, but actually cost more in the long run since more is required to realize stress tolerance benefits. Economic comparisons should be done on a cost per unit area basis.) Confusion concerning biostimulant treatments seems to arise from the fact that many materials are classified as biostimulants but have not performed as advertised. At Virginia Tech we have shown that seaweed extracts, humic acids, triazole fungicides, amino acids, potassium silicate, and most recently, low doses of salicylic acid, have demonstrated biostimulant properties. However, for the two most commonly used biologically active ingredients, seaweed and humic acid, there are different sources and different procedures for extracting these materials from their sources.

The chemical composition of seaweed is determined by the condition in which it is grown. Dr. T.L. Senn from Clemson University has studied the influence of seaweed

products on plants for many years. He indicates that brown seaweed, Ascophyllum nodosum, prepared by alkaline hydrolysis from Norwegian waters is a stable product when subjected to rigid quality control (Senn, 1987). We have found seaweed extracts obtained from a similar latitude in Nova Scotia waters to provide similar biological activity (Schmidt and Zhang, 1997; Zhang and Schmidt, 1997, 1999, 2000a). Hormones such as cytokinins and auxins have been isolated and quantified from these extracts (Sanderson and Jameson, 1986; Sanderson et al., 1987; Crouch and Van Staden, 1993) and may be the active ingredients.

Humic substances can be extracted from soils, peat, coal (leonardite) and lignite in an alkaline solution. These extractions are separated into humic acid and fulvic acid fractions by acidification. Humic acids are precipitated under a pH of 2 or lower. Fulvic acids, soluble at all pHs, have lower molecular weights and are the most biologically active fraction (Prat, 1960). The two main aspects of these humic fractions that have influence of plant growth are the auxin content and ability to chelate certain inorganic nutrients such as iron.

Results from independent research reports should be used to verify the efficacy of commercial biostimulant materials and also to confirm that suppliers are providing formulations with proper dose and application frequency recommendations. Our experience is that frequent, low dose applications are more beneficial than infrequent high dose treatments. One should be aware that most of the biostimulants are hormonal in nature and excess applications could be harmful to plants. For frequent applications we obtained excellent results when 0.5 oz of humic acid, 0.2 oz of seaweed extract or 0.5 oz of triazole fungicide were applied per 1000 sq ft., (Because of labeling laws triazole fungicides will not be packaged with other biostimulants. The superintendent will have to do the blending).

The effect of a single biostimulant application can be expected to decrease gradually with time. There are some indications that better results are obtained when sequential treatments are made and the second year is better than the first year. Monthly applications prior to and during the stress periods (three to six applications per year) should be programmed. Many biostimulant products list the mineral nutrient content in order to qualify in labeling. Sometimes these are listed as auxiliary to the biologically active substances (such as hormones). The "bonus" major nutrients are generally small amounts and contribute little to plant growth and development.

WHAT CAN REASONABLY BE EXPECTED FROM BIOSTIMULANT TREATMENTS? Let's begin with an explanation of how environmental stress damages plant tissues and then discuss what we have observed and documented regarding the ability of biostimulants to diminish these damaging processes. Under favorable conditions molecular oxygen accepts electrons during metabolic processes, producing water as a byproduct. However, under unfavorable conditions this oxygen accepting electron process can be overwhelmed resulting in the production of a number of toxic oxygen species. These toxic oxygen species are often referred to as free radicals and go by names such as superoxide, singlet oxygen, hydrogen peroxide, and the hydroxyl radical. These radicals, left as they are, cause pigment breakdown (bleaching) and major damage to cell walls, mitochondria, and chloroplasts. All of this leads to a loss of photosynthetic efficiency, cell death, and eventually, plant death.

Plants deal with these damaging by-products of metabolism by producing chemical compounds that are appropriately called anti-oxidants. These compounds react with oxygen radicals to produce non-toxic end products such as water and molecular oxygen. Under non-stress conditions enough antioxidants generally are produced to quench or de-toxify these ever-present free radicals. However, any type of environmental stress can greatly increase free radical generation, overwhelming the ability of the antioxidant system to stop their damaging effects. If this happens and the

stress continues, the plant goes into a survival mode that can culminate in semidormancy or plant death. During this high-stress phase, greater levels of the hormone ethylene are produced that signal leaf senescence to proceed and conserve energy reserves in plant growing points (turfgrass crowns). Photosynthesis halts and respiration is greatly reduced, only continuing to a minor extent in the crowns and roots. Higher levels of ethylene almost always coincide with reductions in the growth hormones cytokinins and auxin.

We postulate that pre-treatment with the biostimulant active ingredients seaweed extract and humic acid may change the hormonal balance to favor cytokinins and auxin over ethylene enough so that antioxidant production can continue when stress occurs. In effect the turf may be "triggered" into continuing to grow and protect itself during periods of stress when it normally would begin to shut down.

So, according to our research, the paramount impact that biostimulants have on turfgrass stressed by drought (Zhang and Schmidt 1997, 1999, 2000a, 2000b), salinity (Yan, 1993; Nabati et al., 1994), heat (Zhang et al., 2002a,b; Ervin and Zhang (in progress), cold (Schmidt and Chalmers, 1993; Ervin and Munshaw (in progress)), dollar spot (Zhang et al., 2002c; Ervin and Zhang (in progress)), high UV light intensity (Schmidt and Zhang, 2001; Ervin and Zhang (in progress)), herbicides (Schmidt and Lui, 1993; Zhang et al., 2001; Ervin et al., (in progress)), and nematodes (Sun et al., 1997) is associated with the stimulation of endogenous antioxidant development that protects the plant during the formation of excess free radicals.

For example, many warm and cool season turf areas now are being irrigated with brackish water. As water is lost through evapotranspiration salt accumulates in the soil and plant free radicals will increase due to this salinity stress. The antioxidants stimulated by biostimulant treatments will help offset the toxic influence of the free radicals (Winston, 1990). In addition, we have some evidence that uptake of sodium and chloride is reduced in biostimulated treated grass (Yan, 1993).

Possibly, the tolerance of low soil moisture could be the most evident and beneficial aspect associated with biostimulant treatments of turfgrasses (Zhang and Schmidt, 1999). Biostimulant treated plants retain more moisture than non-treated grasses when subjected to dry soil conditions. Longer periods between irrigations may be programmed and less afternoon syringing to prevent wilting may be needed for biostimulant treated turf. At Virginia Tech we have shown that in addition to treatments with seaweed extracts, humic acid, triazole fungicides or amino acids, applications of potassium silicate (Schmidt et al., 1999), and more recently, low doses of salicylic acid (Schmidt and Zhang 2001) have demonstrated biostimulant properties. However, there are different sources of seaweed and humic acid, and different procedures for extraction of these sources. Also, commercial products differ in their concentrations of amino acids and silicates. Our research indicates that the correct dosage of salicylic acid is an important aspect in obtaining positive results.

Safer use of preemergence herbicides may be obtained with biostimulant treatments (Schmidt and Lui, 1993). Our recent research has shown that applications of biostimulants applied separately or in tank mixtures with postemergence herbicides increased the efficacy of the herbicide (Zhang et al., 2001). This indicated that lower dosages of these herbicides could be employed. We postulate that the biostimulant increased translocation of the herbicide. In addition, we obtained reduced phytotoxicity to non-target plants when a biostimulant was applied in conjunction with certain preemergence or postemegence herbicides (Ervin et al., in progress). The injury reduction apparently was related to the increased antioxidant activity associated with the biostimulant treatment.

Basically, biostimulants enhance plant metabolic activity to condition the plant to tolerate stresses. Therefore, biostimulants have a greater impact when applied prior to

the turf being subjected to an anticipated stress.

# WHAT ABOUT MIXING THE DIFFERENT TYPES OF MATERIALS THAT HAVE BIOSTIMULANT PROPERTIES?

Our research has shown mixing different biostimulating materials to be a good practice in some cases. For example, the combination of a triazole fungicide, humic acid and seaweed extract has provided excellent results (Zhang and Schmidt, 1999). Dosages of each of the materials when blended may be lowered, and yet retain the efficacy of the biostimulant effect and disease control. This combination reduces the need for high triazole fungicide rates to control certain diseases. Higher triazole rates may be detrimental to turfgrasses under certain conditions. The combination of seaweed and humic acid generally has better biostimulanting properties than either material alone. The use of trinexapac-ethyl (Primo) to suppress turfgrass top growth has increased in popularity. We have shown that applications of trinexapac-ethyl have increased endogenous antioxidants, but not root growth as other biostimulant materials have. The addition of seaweed extract with trinexapac-ethyl may encourage the development of roots under stress periods. Our current research has shown that the incorporation of salicylic acid with seaweed and humic acid enhanced biostimulant properties.

#### VIRGINA TECH USED TO ADVOCATE FREQUENT APPLICATIONS OF IRON TO HELP ALLEVIATE TURFGRASS ENVIRONMENTAL STRESSES. IS IRON STILL NEEDED IF BIOSTIMULANTS ARE APPLIED?

Of all the essential micronutrients iron is required by turfgrasses in the largest quantity. Iron is not a part of the chlorophyl structure but it is essential for normal chlorophyll formation. Although iron is abundant in soil it is not readily available to plants. Grasses release mungineic acid that enables iron to be transported across root cell membranes (Fett et al., 1998). In the western part of the United States, where most adapted grasses release muginec acid slowly, iron is tied up with calcium and here the benefits from application of iron fertilizer are most evident. In the eastern part of the United States, where soils tend to be acid, soil iron is readily taken up when the grasses exude mugineic acid that chelates the soil iron to enhance absorption by the grass. Unfortunately, when soils are hot or cold the cool season grasses evidently do not exude this acid sufficiently. During these times (hot summer and cold autumn and winter), iron fertilization of cool season grasses provides a most visible response. With chilling temperatures in the fall, warm season grasses respond positively to iron fertilization (White and Schmidt, 1989). Iron fertilization could be beneficial during unanticipated stress conditions. For this reason, and the quick greening iron provides some biostimulant producers incorporate iron in their product. Others suggest iron be applied in conjunction with their material.

#### HOW FREQUENT SHOULD IRON BE APPLIED?

This depends on how frequently the grass is mowed. Tillers and newly developed leaves are strong sinks for iron (Mori, 1989). Since the newest leaf areas are removed when the grass is mowed much endogenous iron is removed with the grass clippings. To insure optimum iron nutrition of the grass, retreatment of iron should be made every three to four weeks during the stress periods of the cool season grass-growing season. Again, frequent low doses perform better than infrequent heavy doses. Effects of foliar iron applications on cool season grass in late fall when mowing frequency is reduced may be evident for several months (Snyder and Schmidt, 1974).

In autumn, with the onset of chilling temperatures (three to four weeks prior to frost), applications of iron to warm-season turfgrasses has been beneficial. Iron fertilization will help improve the grass's photosynthetic efficiency and help maintain color after light frosts (White and Schmidt, 1989; Schmidt and Chalmers, 1993).

### WHAT TYPES OF IRON SHOULD BE APPLIED?

Even though chelated iron is somewhat expensive most superintendents prefer this

form of iron since this type is more readily available to the plant from soil. There are several methods used to chelate iron and not all provide equal performance. However, the less expensive non-chelated iron (ferrous sulfate) can become chelated when applied with humic acid. Generally speaking, we have obtained good results when most ferrous iron products are applied to foliage. The effectiveness of an iron source can be ascertained readily by the rapid enhancement of green color when applied to the turf during an appropriate stress period.

### HOW MUCH IRON SHOULD BE APPLIED AT ANY ONE TIME?

Based on our experiences one pound of iron per acre (1 kg per Ha) per application is a suggested rate. That would be two ounces of a 20% iron material per 1000 ft2 (60 g per 100 m2). However, some superintendents have obtained excellent results when applying two to three times this much to bermudagrass in early autumn before the chlorophyll is destroyed by frost. In fact, these heavy applications of iron helped preserve the bermudagrass color into mid fall in spite of several light frosts.

## DO BIOSTIMULANTS AFFECT TURFGRASS FERTILIZATION PROGRAMS?

Biostimulants, even those containing some mineral fertilizers, do not necessarily supply all the essential nutrients in the quantities a plant needs. Their main function is to condition the grass to tolerate environmental stresses. Therefore, biostimulants should be used in conjunction with proper mineral fertilization. We do feel that biostimulants enhance the effectiveness of conventional fertilizers. Research indicates that less mineral fertilizers are required when biostimulants are used (Schmidt, 1992; Frankenberger and Arshad, 1995). This has a practical and environmental impact. An example of the practical aspect is that applying less nitrogen than the conventional rates to the newer creeping bentgrasses results in reduced thatch development and possible subsequent deterioration of the turf associated with heavy nitrogen fertilization. Biostimulant treatments should be beneficial when used in conjunction with the "spoon feeding" technique of fertilizing bentgrass putting greens currently advocated by some superintendents. This is especially true when summer application of nitrogen is scheduled. With the reduced application of mineral nutrients, the potential of soil-water contamination is reduced, resulting in good environmental practices.

### CONCLUSIONS

Research on biostimulants permits advancement in turfgrass nutrition. It has been established that the mineral content between plants of the same cultivar with different growth responses is relatively small. Mineral nutrition is poorly correlated with tolerance to stresses. However, with the understanding of the impact that biologically active materials have on turfgrass metabolism, turfgrasses may be better conditioned to tolerate environmental stress. Utilizing this knowledge provides an additional tool to produce quality turf grown under adverse environments.

### LITERATURE CITED

Allen, V.A.G. 2000. Forages for Grazing Animals. In: A spectrum of achievements in Agronomy: Women Fellows of the Tri-Societies. ASA Special Publication No. 62. American Soc. of Agron, Crop Science Society of America, and Soil Science of America, Madison WI. pp. 65-69.

Crouch, I.J. and J. Van Staden. 1993. Evidence for the presence of plant growth regulators in commercial seaweed products. Plant Growth Regul. 13:21-29. Doyle, Sir Arthur Conan. 1987. The adventures of Sherlock Holmes. The Reader's Digest Assoc. Pleasantville, NY.

Ervin, E.H. and G. Munshaw. 200x (in progress). Hormone and lipid changes associated with bermudagrass cultivar acclimation and freezing tolerance.

Ervin, E.H. and X. Zhang. 200x (in progress). Influence of metabolic enhancers on creeping bentgrass rootzone heat tolerance.

Ervin, E.H. and X. Zhang. 200x (in progress). Influence of metabolic enhancers on nonfungicidal

and fungicidal control of dollar spot on creeping bentgrass.

Ervin, E.H. and X. Zhang. 200x (in progress). Improving Kentucky bluegrass UV tolerance with metabolic enhancers.

Ervin, E.H., X. Zhang, and R.E. Schmidt. 200x (in progress). Improving herbicidal selective suppression of bermudagrass with metabolic enhancers.

Fett, J.P., K. LeVar and M.L. Guerinot. 1989. Soil Microoganisms and iron uptake by higher plants. In:Metal ions in biological systems. Vol. 35. Sigel, A and H. Sigel (eds.) Marcel Dekker Inc. New York.

Frankenberger, W.T. Jr. and M. Arshad. 1995. Phytohormones in Soils. Marcel Dekker, New York, Basel, Hong Kong.

Mori, S. 1998. Iron transport in Gramenicease plants. In: Metal ions in biological systems. Vol. 35. Sigel a. and H. Sigel (eds.) Marcel Dekker Inc. New York

Nabati, D.A., R.E. Schmidt and D.J. Parish. 1994. Alleviation of salinity stress in Kentucky bluegrass by plant growth regulators and iron. Crop Sci. 43: 198-202.

Prat, S. 1960. Distribution of the humus substance fractions in plants. Biol. Plant (Prague) 2: 308-312.

Sanderson, K.J. and P.E. Jameson. 1986. The cytokinins in a liquid seaweed extract: could they be the active ingredients? Acta Horticulturae 179:113-116.

Sanderson, K.J., P.E. Jameson, and J.A. Zabkiewicz. 1987. Auxin in a seaweed extract: identification and quantitation of indole-3-acetic acid by gas chromatography-mass spectrometry. J. Plant Physiol. 129:363-367.

Schmidt, R.E. 1992. Poultry by-products used in conjunction with biostimulants for turfgrass growth. In: J.P. Blake, J.O. Donald, and P.H. Patterson (eds.). Proceedings of the National Poultry Waste Symposium. Auburn University, AL. ISBN: 0-9627682-6-3. Schmidt, R.E. and D.R. Chalmers 1993. Late summer to early fall applications of fertilizer and biostimulants on bermudagrass. In: R.N. Carrow, N.E. Christians, and R.C. Shearman (eds.) International turfgrass Society Research Journal 7: 715-721. Intertec Publishing Corp, Overland Park, KS

Schmidt, R.E. and Wang-Juan Lui. 1993 Pendimethalin Influence on seedlingly Kentucky bluegrass developed from plant growth reglator-treated seed. In: Carrow, R. N. N.E. Christian, R.C. Shearman and M. S. Welterien (eds.) International Turfgrass Society Research Journal 7: 708-714. Intertec Publishing Corp., Overland, KS Schmidt, R.E. and X. Zhang. 1997. Influence of seaweed on growth and stress tolerance of grasses. In: M.J. Williams (ed.) Proc. Am. Forage Grassl. Council. Fort Worth. TX. p. 158.

Schmidt, R.E. and X. Zhang. 2001. Alleviation of photochemical activity decline of turfgrasses exposed to soil moisture stress or UV radiation. In: K. Cary (ed) International Turfgrass Society Research Journal 9:340-346.

Schmidt, R.E., X. Zhang, And D.R. Chalmers. 1999. Response of photosynthesis and superoxidide dismutase to silica applied to creeping bentgrass grownunder two fertility levels. Journal of Plant Nutrition. 22: 1763-1773.

Senn, T.L., 1987. Seaweed and Plant Growth. Clemson University, Clemson, SC. Snyder, V. and R.E. Schmidt. 1974. Nitrogen and iron fertilization of bentgrass. In: E. C. Roberts (ed.) Proceedings of the Second International Turfgrass Research Conference. Blacksburg, Va. 7:176-184.

Sun, H., R.E. Schmidt and J.D. Eisenback 1997. The effect of seaweed concentrate on the growth of nematode-infected bent grown under low soil moisture. In: P.M. Martin and A. E. Bauman (eds.) International Turfgrass Society Research Journal. 8: 1336-1342.

White, R.H. and R.E. Schmidt. 1989. Bermudagrass responses to chilling temperatures as influenced by iron and benzyladenine. Croop Sci. 768-773.

Winston, G.W. 1990. Physiochemical basis for free radical formation in cells production

and defense. In: Alsher. R. G. and J. R. Cumming (eds.). Stress responses in plant. Adaptation and acclimation mechanisms. pp. 57-86. Wiley-Liss, New York, NY. Yan, J. 1993. Influence of plant growth regulators on turfgrass polar lipid composition, tolerance to drought and salinity stresses and nutrient efficiency. PhD dissertation. Dept. Crop and Soil Environmental Sciences, Virginia Polytechnic Institute and State University, Blacksburg, VA.

Zhang, X. 1997. Influence of plant growth regulators on turfgrass growth, antioxidant status, and drought tolerance. PhD dissertation . Department Crop and Soil Environmental Sciences, Virginia Polytechnic Institute and State University, Blacksburg, VA.

Zhang X. and R.E. Schmidt. 1997. The impact of growth regulators on the alphatocopherol status in water-stressed Poa pratensis L. In Martin P.M. and A. E. Bauman (eds.) International Turfgrass Society Research Journal 8:1364-1371.

Zhang X. and R.E. Schmidt. 1999. Antioxidant response to hormane-containing products in Kentucky bluegrass subjected to drought. Crop Science 39:545-551. Zhang, X. and R.E. Schmidt. 2000a. Application of Trinexapac-ethyl and Proiconazole enhances photochemical activity in creeping bentgrass (Agrostis stoloniferous var. palustris). J. Amer. Soc. Hort 125:47-51.

Zhang, X. and R.E. Schmidt. 2000b. Hormone-containing products' impact on antioxidant status of tall fescue and creeping bentgrass subjected to drought. Crop Sci. 40:1344-1348.

Zhang, X., R.E. Schmidt, and P.L. Hipkins. 2001. The influence of selected PGRs on postemergence herbicide efficacy. In: K. Cary (ed) International Turfgrass Society Research Journal. 9:1056-1061.

Zhang, X., E.H. Ervin, and R.E. Schmidt. 2002a (in review). Plant growth regulator enhancement of Kentucky bluegrass sod recovery following heating. Crop Sci. Zhang, X., E.H. Ervin, and R.E. Schmidt. 2002b (in review). Plant growth regulator enhancement of tall fescue sod recovery following heating. HortScience. Zhang, X., E.H. Ervin, and R.E. Schmidt. 2002c. (in review). Physiological effects of liquid applications of a seaweed extract and a humic acid on creeping bentgrass (Agrostis palustris Huds. A.). J. Am Soc. Hort. Sci.