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University of California (Davis) scientists discuss the development of a model plant system that allows for the rapid screening of foliar chemicals. This system has been used to determine the relative efficacy and efficiency of a range of widely available foliar zinc products. Zinc was selected for these studies as it is the most widely used micronutrient in US agriculture but has largely been ignored in turfgrass production.

Volume 7, Number 12
June 15, 2008

PURPOSE

The purpose of *USGA Turfgrass and Environmental Research Online* is to effectively communicate the results of research projects funded under USGA's Turfgrass and Environmental Research Program to all who can benefit from such knowledge. Since 1983, the USGA has funded more than 350 projects at a cost of $29 million. The private, non-profit research program provides funding opportunities to university faculty interested in working on environmental and turf management problems affecting golf courses. The outstanding playing conditions of today's golf courses are a direct result of ***using science to benefit golf***.

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Development of a Model System to Test Foliar Fertilizers for Use in the Turfgrass Industry

Patrick H. Brown and David Burger

SUMMARY

Foliar fertilization has become a standard practice of many turfgrass managers. However, our understanding of this important class of products is remarkably poor, and for the majority of foliar fertilizers, there is very little information on their effectiveness. This lack of information is a consequence of both the great difficulty in conducting research with foliar fertilizers and a general paucity of research in micronutrients in turfgrass production. Here we discuss the development of a model plant system that allows for the rapid screening of foliar chemicals. This system has been used to determine the relative efficacy and efficiency of a range of widely available foliar zinc (Zn) products. Zinc was selected for these studies as it is the most widely used micronutrient in US agriculture but has largely been ignored in turfgrass production. Additional experiments to develop a model grass system to test a range of foliar products on turfgrass species are underway. The key observations and findings are:

- Foliar fertilizers are a critical component of modern turf management and yet there is a paucity of information on their use. Foliar Zn nutrition has not been examined in turfgrass.
- The model plant test system developed here is a powerful approach to screening large numbers of materials under controlled conditions and provides essential preliminary information to guide subsequent field testing.
- Foliar Zn formulations vary greatly in their efficacy and efficiency. The solubility of the chemical form of zinc in the fertilizer product is a primary determinant of efficiency of the product.
- The specialty foliar Zn complexed and chelated products sold as liquids vary from moderate to excellent efficacy and were generally more efficient than non-complexed Zn salts.
- Zinc has been overlooked as a component of turfgrass fertility management and further research and field testing is warranted. Foliar fertilizers can play an important role in Zn management, however not all foliar fertilizers are equally effective.

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USGA Turfgrass and Environmental Research Online 7(12):1-10.
TGIF Record Number: 137274

The Essential Nutrients for Turgrass Growth: Where's the Zinc?

Fourteen elements (excluding C, H, O) are known to be essential for plant growth: N, P, K, Ca, Mg, S, Fe, Cu, Mn, Zn, Mo, Cl and Ni. While the concentration of these essential elements in plant tissues varies by a ratio of more than 10 million to 1 from nitrogen to nickel, all are essential and plant growth will decline if they are not present in adequate amounts.

In turfgrass management, however, only N, P, K, S and Fe are routinely applied; Ca and Mg are occasionally applied; and the micronutrients Zn, Mn, B, Cl, Ni are rarely applied as a targeted treatment (7, 13). The lack of targeted application of Zn, in particular, is striking given that zinc is the most widely used micronutrient in US agriculture (Figure 1) and is the most common micronutrient deficiency worldwide (3). The cereal grasses rice, wheat, and corn are particularly sensitive to Zn deficiency and the majority of all cereal production now receives Zn as a routine practice (1). Over the past decade, there has also been a prominent and growing use of Zn in agronomic and horticultural crops across the US.

Zinc performs a wide array of functions in plants (2) including functioning as an essential component of gene transcription and translation, a structural and functional component of a wide range of enzymes including many associated with photosynthesis and membrane stability, and a critical element for temperature, light, and heat stress tolerance (4). While all of these functions are critical for turfgrass growth and development, Hull (7), has further emphasized the importance of Zn for processes of specific importance to turf production including waterlogging tolerance, high temperature and light stress resistance, maintenance of photosynthesis in warm climate species and protection against fungal diseases.

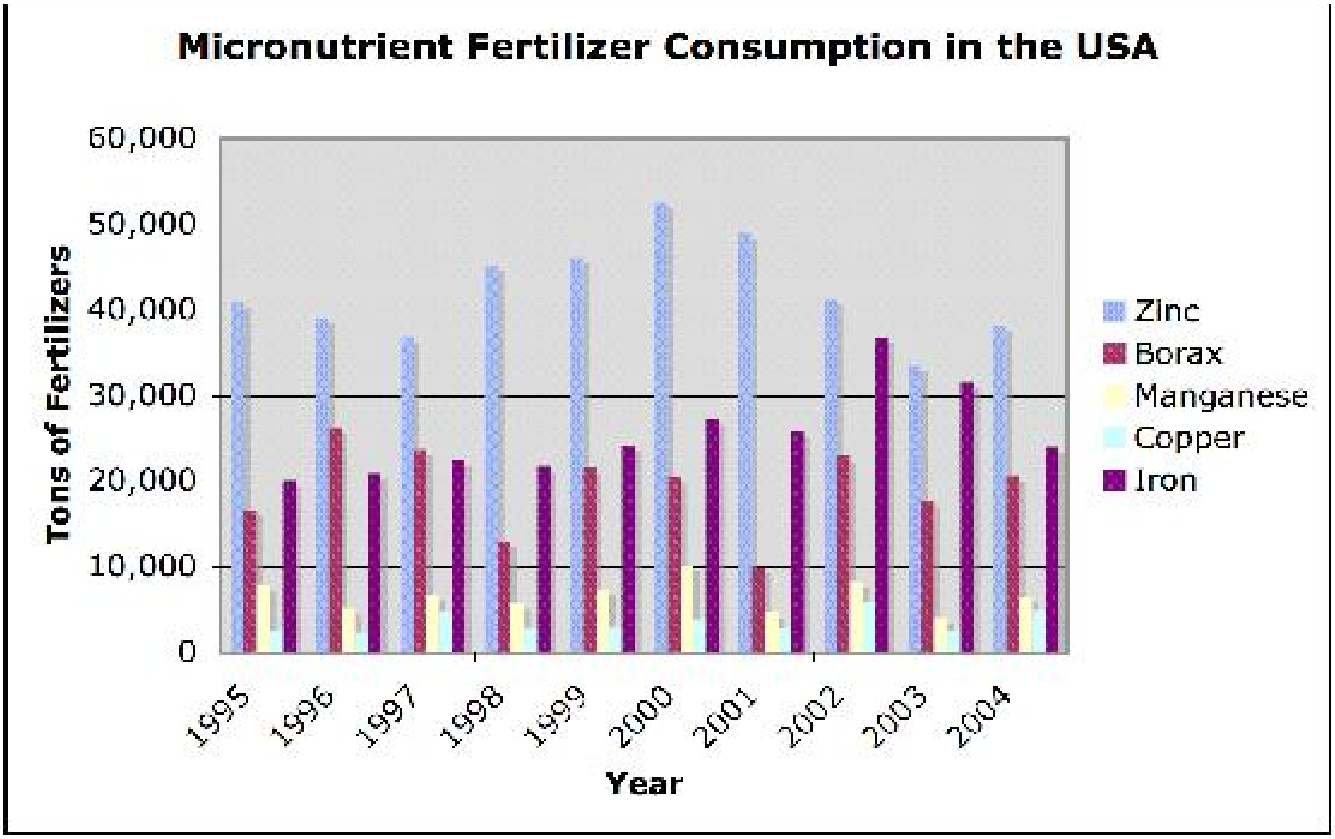

Figure 1. Total Micronutrient consumption for select micronutrients over the period 1995 to 2004. This data is sourced from fertilizer tonnage sales reported to respective state agencies and Plant Food Control Officers in each state. Values shown are gross total nutrient consumed and do not consider form of the element or respective nutrient solubility. Micronutrients incorporated into bulk blended, finished fertilizers and other specialty fertilizers are not included in these data. Data courtesy of the American Association of Plant Food Control Agents (AAPFCO).

In agricultural crops, Zn deficiency occurs most prominently in high pH soils, but also occurs in soils with high levels of reactive organic matter and in coarse-textured, sandy soils of low mineral nutrient content. Zinc deficiency in US agriculture is observed more frequently in the predominantly calcareous soils of the western and mountain states, the north central region, and in the acid, coarse textured soils of the southeastern and southern states like Florida. Globally, it is the grass species, wheat, rice, and corn that utilize the greatest amounts of fertilizer zinc. Zinc seed and starter fertilizers are now utilized in much of the corn production in the US and in a large percentage of global rice and wheat production (1).

Environmental conditions and fertilizer management can also result in Zn deficiency. In corn and in a range of horticultural species, Zn deficiency can occur in early spring when plant growth has initiated, but soil temperatures are low and/or the soil is saturated with water. In rice, lack of soil oxygen, excess levels of soil Fe can induce Zn deficiencies while in many species, high temperatures and high light levels can increase the metabolic demand for Zn and increase the impact of a latent deficiency (4). The choice of nitrogen source can also exacerbate the occurrence of Zn deficiencies with the use of coated urea materials having a significant negative effect on Zn deficiency in rice (11). The application of excess phosphorus is also well known to induce Zn deficiency in a wide range of cropping systems (9).

The State of Knowledge Regarding Zinc in Turfgrass

The functions of Zn in plants, its specific importance for grass species and the conditions under which modern greens are managed all sug-

Figure 2. Zinc deficiency in corn showing interveinal chlorosis, bleaching and shortening of internodes resulting in stunted compact growth. (photo courtesy of J.E.Sawyer http://extension.agron.iastate.edu/soil-fertility/photos/photossdef.html)

gest that Zn nutrition should be of concern for golf course superintendents. There are, however, very few scientific reports of turfgrass response to fertilizer Zn additions (13) and little to no information on the identification and correction of Zn deficiencies can be found in the widely cited turfgrass management publications (8, 9, 10). This lack of published proof of a benefit of fertilizer Zn on turf production, however, should not be interpreted as proof that there is no need for fertilizer zinc (7).

Though the experimental data is lacking, it is generally accepted that Zn is required in turfgrass leaves at concentrations of from 30 to 80 parts per million (microgram per gram dry matter) depending upon cultivar (7). The true relevance of these 'typical' concentrations for optimal plant productivity remains unknown. In agricultural species, symptoms of Zn deficiency include reduced growth and vigor, often associates with a shortening of the internodes (distance between leaves) resulting in a shortened brushy appearance of emerging tillers (Figure 2). Plants may show yellowing and interveinal chlorosis (Figure 3) , patchy necrosis or leaf bleaching (Figure 4). Occurrence of a deficiency in a field or turf area can often be irregular, corresponding to changes in soil type, waterlogging, or localized high heat and high light stress.

While visual symptoms can be used to help identify a nutrient deficiency, it should be recognized that the goal of a good agronomist, horticulturalist, or superintendent is to avoid the occurrence of any deficiency. Once a deficiency is severe enough to be identified by visual symptoms, invariably growth, aesthetic appearance, and stress resistance have all been compromised. To ensure optimal productivity, it is essential that nutrients removed from the ecosystem in clippings or harvested yield, leaching, and erosion be replaced.

The likelihood that an element such as Zn will become deficient can also be determined with the help of soil and plant testing. While specific guidelines do not exist for the interpretation of soil and plant tests in turfgrass, there are certain conditions including high soil pH (>8), high sand content, and declining tissue Zn concentrations that are known to exacerbate the occurrence of Zn deficiency. Given the lack of any alternative, the tissue and soil standards established for corn may be used as a guide for Zn management in turfgrass.

Given the known functions of Zn in plants, the soil and environmental conditions that result in Zn deficiency and the widespread occurrence of Zn deficiencies in US agriculture, it is perhaps surprising that Zn deficiency has not been more widely reported in the US turfgrass industry. This may in part be a consequence of the lack of clear description of Zn deficiency in turfgrass species, particularly at marginal levels of deficiency, and is complicated further by interactive effects of heat stress, cold temperatures, and water logging on the expression of Zn deficiency.

Zinc is well known to have beneficial effects on plant tolerance to stress conditions (4), so it is probably not a coincidence that many of the foliar products sold on the basis of their 'biostimulant' or 'stress protection' function actually contain significant levels of Zn in addition to a

Material Name[1]	Manufacturer or Distributor[2]	Concentration (ppm)[3]	Overall Ranking[4]	Comments
EleMax Super Zinc FL 1-0-0	Yara	500	1	40% Zn as zinc oxide. Miscible in water, solubility limited.
Neutral Zinc	MontereyAgChem	400	1	52% zinc oxide and sulfate.
RNA Microphos	RNA Corporation	400	3	52% Zn as phosphate/oxide mix ture. Miscible in water, solubility limited.
Zinc Fulvic Acid	Wilbur Ellis	400	4	7% zinc fulvic acid complex. Moderate effectiveness
Zinc sulfate		400	5	36% zinc sulfate.
Krystal Klear Zn	Lidochem Inc.	400	6	9% synthetic chelated Zn. Moderate and consistent response.
Zinc Lignosulfonate	Wilbur Ellis	400	6.5	7 - 10% zinc sulfate ignosulfonate. Moderate effectiveness, some variabili ty in response.
Bio-link ZN	Westbridge	400	6.6	8% Zn, hydroxy-carboxylic, amino acid complex. Consistent and effective.
RNA Zinc nitrate		400	7.0	
RNA Microphos	RNA Corporation	5,000	7.2	52% Zn as phosphate/oxide mix ture. Miscible in water, solubility limited.
BioMin Zn	JH Biotech	400	7.3	7% zinc sulfate, citric acid, glycine. Consistent and effective.
Zn Metalosate	Albion	400	7.3	7% Metalosate amino complexed Zn. Consistent and very effective.
BioNutrient Zn+	Wilbur Ellis	400	7.3	8% zinc. Consistent and very effective.
Zinc EDTA		400	8	10% EDTA complexed Zn. Very effective and very consistent.
Neutral Zinc	Monterey AgChem	1,860	8	52% zinc oxide and sulfate.
Zinc sulfate		1,500	8	36% zinc sulfate. Good effectiveness.
Foli-GroNZn	Wilbur Ellis	400	8.4	5% Zn as Zn nitrate with urea and urea ammonium nitrate. Very effective and very consistent.
FloratineZicron-F	FloratineBiosci	400	8.6	6% zinc carbon complex. Very effective and consistent.
Zinc Manniplex	Brandt Chemicals	400	9	7% zinc carbon complex. Highly effective very consistent response.

[1] 1 = no significant difference from water control; 2-4 = measurable but small increase in tissue Zn; 5-7 = consistent and significant increase in tissue Zn, good effectiveness; 8-10 = consistent and very significant increase in tissue Zn, superior effectiveness.

[2] Mention of a product trade name or commercial enterprise does not imply endorsement of this product or commercial enterprise by the author or the University of California, Davis. If a distributor is not identified then the product can be assumed to be widely available.

[3] Materials were applied to foliage only, at either a standard 400 ppm concentration in spray solution . Where 400 ppm Zn was not the approximate field application rate, experiments were repeated at the higher field rate. Experiments were conducted in a non-grass system and products and rates listed may not be labeled or appropriate for turfgrass use.

[4] Products shown with numerical rankings that differ by 0.9 or greater are significantly different at the 0.05% level of significance.

Table 1. Ranking of various foliar zinc products in common commercial use

host of other compounds of less certain physiological function.

With the widespread adoption of the USGA sand green, the development of higher density, faster growing turfgrass cultivars, the enhanced stress caused by lower cutting heights and the potential effects of global warming, Zn management in turf should be re-examined.

Supply of Zinc to Turf and the Evidence for the Use of Foliar Fertilizers

Whereas the majority of Zn fertilization in agronomic crops occurs as a soil or seed application, increasingly in horticultural and other high value species, Zn is applied as a foliar fertilizer. Foliar chemicals are used widely in horticulture for a number of reasons, the most common of which is a perceived advantage in control of application timing to match crop demand (3). Foliars are also superior for the correction of deficiencies in high pH, Zn-fixing soils and may be advantageous in supplementing excessive nutrient demand during rapid plant growth (spring and early summer flushes), during periods of impaired nutrient uptake due to low temperature or soil flooding (early spring), or as a consequence of reduced nutrient uptake during periods of limited root growth (summer stress in cool-season grasses).

While foliar fertilizers have gained acceptance in turfgrass management, our understanding of the principles that determine the efficacy of foliar nutrients is extremely poor. The factors that ultimately determine the nutrient use efficiency (NUE) of a foliar formulation are quite complicated and are dependent upon many physical, biological, and environmental factors including:

The physicochemical characteristics of the formulation:

- Effect of drying time, crystallization, solubility and deposition uniformity (concentration) on the quantity and distribution of applied nutrient element on the leaf surface
- Effect of chemical formulation and physical properties on penetration and distribution of nutrient element within sub-cuticular tissues
- Effect of formulation on biological utilization of absorbed element
- Effect of formulation on short- and long-distance transport of the element away from the site of application.

Environmental and biological condition present at application:

- Leaf temperature, relative humidity, wind speed, sun exposure, application techniques
- Species, leaf age, leaf structure and composition, and leaf damage
- Drought, disease, nutrient status, phenological stage
- Biological complexation, transport processes, compartmentation, sequestration, etc.

The great complexity of factors that influence the effectiveness of a foliar fertilizer makes the conduct of field experiments extremely difficult to perform and interpret, and misleading results can easily be obtained. Studies of foliar Zn products are particularly difficult to interpret since Zn has a tendency to adhere irreversibly to leaf surfaces making it impossible to distinguish between surface adhering (non-functional) Zn and functional Zn that has entered the leaf. To overcome these problems and to provide a robust and interpretable test of fertilizer, test systems are needed that avoid the complications of environ-

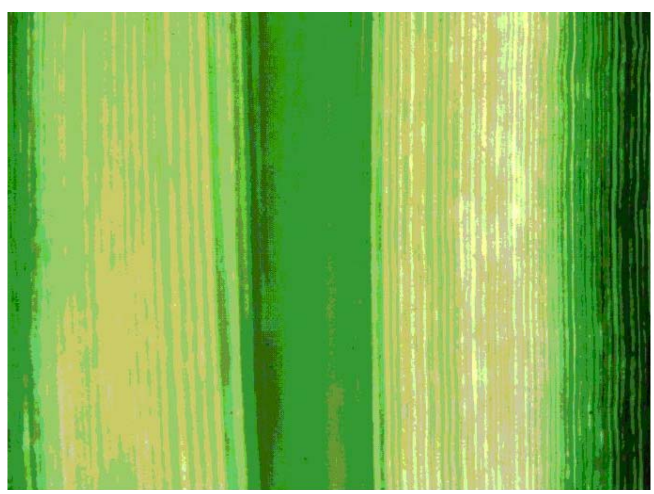

Figure 3. Zinc deficiency induced interveinal chlorosis and bleaching in warm season grass species.

Figure 4. Zinc deficiency in wheat showing characteristic necrosis and chlorosis. (Photograph courtesy of CIMMYT http://wheatdoctor.cimmyt. org/index.php?option=com_easygallery&act=photos&cid=271&Itemid=33)

ment and Zn contamination while simultaneously allowing for a determination of the degree to which the applied product is absorbed by the leaf surface, transported within the plant, and utilized for metabolic activities.

In the following, we describe the development of two model test systems to address these issues. The first of these systems uses a non-grass species (*Arabidopsis thaliana*) that is the most widely used species in plant nutritional, biochemical, and genetic research. This species is selected as it has a short 45-60 day life cycle (cultivar dependent) and a very distinct vegetative/floral transition period which allows for the application of materials to leaf tissue with subsequent recovery of the applied element in the floral tissues thus avoiding the potential for contamination. The small size of the plant also allows for large numbers of replications to be conducted.

In the second model system, we utilize annual bluegrass (*Poa annua* L.) and creeping bentgrass (*Agrostis stolonifera* L.) grown in an artificial solid media and use a repetitive cutting approach to determine Zn uptake and transport rates.

Materials and Methods

Model System 1

Arabidopsis thaliana seeds were planted in trays of eight, 1 inch x 1 inch seedling pots in a soil mixture consisting of 80% fine quartz sand, 20% potting soil mixture. Each tray represents one replicate and all treatments were replicated at least 6 times in each experiment. The entire experiment was then replicated 6 times.

A total of 18 common commercial inorganic salt and liquid zinc formulations were tested during the 6 replicate experiments. Within a single experiment, all treatments were replicated at least 5 times with a total of at least 30 plants. The number of replicate experiments, however, varied with treatment from a single replicate experiment (field rate applications) to as many as 6 replicate experiments for some materials (control, 400 ppm Zn sulfate, and 400 ppm NZn).

Control, 400 ppm Zn sulfate and 400 ppm NZn treatments were included in all trials to represent non-effective, intermediate, and highly effective products and to provide for internal standardization across all experiments. Rankings of each material in comparison with these standards and in comparison with other materials included in the same experiment, were then integrated into a final summary table (Table 1).

Each formulation was applied at day 25-35. At this time, all plants were in the vegetative stage of growth, and any early reproductive tissues were removed. Spray applications were applied to leaves to runoff using a controlled mist application with the addition of Silwet L-77

Figure 5. Model growth system designed for testing of foliar fertilizer and non-fertilizer products. System allows for repeated cutting and resampling of each 1.5 x 1.5-inch experimental unit.

spreader. The soil surface was protected from direct contamination with plastic and lanolin barriers. Independent tests using the tracer ion, rubidium, confirmed that no foliar spray materials entered the soil.

Two experimental approaches were used: 1) application of all materials at a 400 ppm Zn in the final application solution, or 2) application of the material at the specified field application rate.

Fourteen to 18 days after spray application as plants commenced senescence, reproductive tissues were collected and analyzed. The leaf tissue that had received the foliar spray was not included, as these leaves would have been contaminated with non-functional residual Zn. The structure of the *Arabidopsis* plant, growth conditions and the manner in which the experiments were conducted make direct contamination of reproductive tissues by leaf-applied Zn extremely unlikely. Increases in tissue Zn as a result of foliar application observed in these trials represent Zn that has been both absorbed by leaves and transported to the reproductive tissue.

<u>Model System 2</u>

The following system is under development and has not yet been utilized for trials of foliar fertilizers.

Seeds of annual bluegrass (*Poa annua* L.) and creeping bentgrass (*Agrostis stolonifera* L.) were placed on the surface of Oasis® Horticube® Growing Medium blocks that measured 3.8 cm wide x 3.8 cm deep x 1.3 cm tall. The blocks had been washed in running deionized water for at least 60 minutes and drained via vacuum before use. Once seeds had been sown, the blocks were placed in 7.5 cm x 7.5 cm x 10 cm tall boxes and 50 ml of the test solution was added. Lids were placed on the Magenta box and placed in an incubator kept at 20°C with a 16/8 hour light/dark photoperiod. An example of this system is provided in Figure 5.

Results and Discussion

Results of the six replicate experiments are provided in Table 1. The data shown represents an integrated assessment of the ranking of each material in contrast to the water (control), 400 ppm zinc sulfate (intermediate effectiveness) and 400 ppm NZn (high effectiveness) standards. Results illustrate that the efficacy of spray materials varies significantly between materials and is strongly influenced by concentration of Zn present in the spray solution.

Solubility Matters

The relative efficiency with which a specific product enhances Zn in the model plant can be determined from experiments in which the materials were provided at a standard concentration of 400 ppm. Here we selected 400 ppm as this standard value as it represents the application rate used in the majority of the liquid foliar formulations. Using these criteria, it can be seen that materials that are largely insoluble miscible suspensions of Zn oxides and phosphates including EleMax, Neutral Zn, RNA Microphos, are ineffective or only marginally effective at the 400 ppm rate. These materials all result in the deposit of a characterisitic white powder on the surface of the leaves which is unacceptable for turfgrass applications.

Among the liquid foliar materials, all were effective at significantly increasing tissue Zn concentrations. Efficacy varied from moderate for Zn sulfate- and Zn lignosulfonate-based materials to good (KrystalKlear, Bio-link), and very good (BioMin, Metalosate, BioNutrient) for the materials containing amino acids and other synthetic or natural complexers. Several sugar- and polysaccharide-based Zn formulations (Floratine Zicron-F and Brandt Manniplex Zn) were shown to be consistently superior across all experiments. Additionally, NZn, which does not contain any organic complex or chelate material, also performed at a superior level.

Efficiency Does Not Equal Efficacy

Several of the foliar materials tested here utilize field application rates that are substantially higher than 400 ppm. These materials are all inorganic forms of Zn (zinc sulfate, zinc oxide, zinc phosphate, and mixes thereof) that are generally not provided as liquids (unless solubilized by a vendor), and none contain any putative chelator or complexing molecule. Field rates of these materials range from 1,500 to 5,000 ppm or 4- to 12-fold higher than the 400 ppm typically used for specialty liquids. On a cost basis, these materials may still be cheaper than the liquid formulations even when applied at substantially higher rates. When applied at field rates, however, all of these materials improved performance.

Zinc Microphos, a Zn phosphate/oxide-based material improved significantly when provided at 5,000 ppm, however it was still deemed an unacceptable product for turfgrass use as it left a heavy white precipitate on the leaf surface that persisted for several days. Both Neutral Zn and Zn sulfate improved dramatically in performance when concentration was increased from 400 ppm to 1,860 and 1,500 ppm, respectively. The improvement in performance of Neutral Zn might have been a consequence of the zinc sulfate contained in that product.

The Bottom Line

The development of this model system has allowed us to contrast 18 materials in a series of 6 experiments over a 6-month period and has provided reproducible and valuable results. This could not have been accomplished in a field setting and illustrates the utility of this approach. These results must be considered in light of the fact that experiments were not conducted on turfgrass under playing conditions, and we cannot not guarantee that identical results will be achieved under 'real' conditions.

However, on the basis of literature and our experience, it is expected that materials ranked superior in these trials have the greatest potential efficacy, and we hypothesize that these superior materials will never be less effective than those materials ranked lower in the accompanying table. We are now developing a model system based upon grass species and will be replicating this Zn trial and commencing additional trials with Fe and other non-fertilizer products in the coming months.

Results of these trials suggest that many of the foliar Zn materials available for use in the marketplace work to varying degrees and that many of the higher priced liquid products, especially those 'complexed' with sugar and polysaccharide molecules are highly effective.

While it is apparent from this research that the use of a heavy dose of an inorganic, low solubility product can result in effective supply of Zn to plants at a competitive price, managers must also consider issues of ease of use, aesthetics, and be concerned with the total amount of Zn that is added to the environment.

Given the importance of Zn to all agricultural cereals and the conditions under which modern turfgrass is managed, it would be surprising if Zn deficiency is not more widely prevalent in the turfgrass industry than is currently recognized. The presence of substantial amounts of Zn in many foliar blends including 'biostimulant' and 'stress response' materials is perhaps the clearest evidence that there could be benefit to the turgrass industry of a targeted study of the management of Zn in turfgrass productivity.

Acknowledgements

The authors wish to thank USGA's Turfgrass and Environmental Research Program for financial support of this project.

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