1990

Creeping bentgrass response to increasing DTPA-extractable zinc levels in modified soil

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Creeping bentgrass response to increasing DTPA-extractable zinc levels in modified soil

by

Grant Thomas Spear

A Thesis Submitted to the
Graduate Faculty in Partial Fulfillment of the
Requirements for the Degree of
MASTER OF SCIENCE

r: Horticulture

State University
Ames, Iowa

1990
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I. INTRODUCTION

Levels as high as 53 mg DTPA-extractable Zn·kg−1 soil have been observed in samples taken from golf course greens in Iowa. Soil test lab reports that accompany these results state that this is an excessive level of Zn for creeping bentgrass (Agrostis palustris Huds.). Interpretations of Zn test results vary greatly among laboratories and there is little information in the literature on which to base these interpretations.

Native soils surrounding these greens often have much lower available Zn levels than the greens. Fungicide applications to the greens may have caused a Zn buildup since fungicides like dithane, zineb and others contain Zn (Daniel and Freeborg, 1979). The application of sewage sludge and micronutrient-containing fertilizers on greens may also have caused Zn levels to increase. Since the solubility of Zn decreases 100-fold for each unit increase in pH, acidification of greens with elemental sulfur could also increase Zn availability (Lindsay, 1971).

Few studies have been conducted on micronutrient toxicity in turfgrasses. Most micronutrient studies involving grasses have focused on the effect of sewage sludge disposal onto forages. Managers of high maintenance turfgrass areas are increasingly concerned about potential hazards of these materials in fertilizers and fungicides.

The objectives of these studies are (a) to observe changes in levels of available Zn in an 8:1:1 (sand:soil:peat) soil mix in response to varying levels of applied Zn, (b) to observe changes in creeping bentgrass tissue levels of Zn in response to varying levels of applied Zn, (c) to
observe the response of creeping bentgrass cultivars to Zn applications and (d) to investigate toxicity of Zn to creeping bentgrass.
II. LITERATURE REVIEW

A. Zn in Plants

Zinc is available to plants as a divalent metallic cation (Zn$^{2+}$). The diverse roles of the metal in plants are still poorly understood. Zinc promotes the synthesis of cytochrome C and is present in phosphodiesterase, carbonic anhydrase, superoxidedismutase, RNA polymerase and the dehydrogenase enzymes: alcohol, pyridine nucleotide, glucose-6-phosphate and triose phosphate (Marschner, 1986; Tisdale et al., 1985). Although it is not a component of chlorophyll, Zn is required for chlorophyll synthesis (Daniel and Freeborg, 1979). Tsui (1948) concluded that Zn is required in tomato for the synthesis of tryptophan, a precursor of the auxin Indole-3-acetic acid. Skoog (1940) concluded that Zn is responsible for maintaining auxin activity but not for auxin synthesis. Ribosomes of plant cells contain Zn which is essential for their structural integrity (Marschner, 1986). Boehle and Lindsay (1969) reported that 15-80 mg Zn·kg$^{-1}$ tissue is sufficient for grass. An average Zn level from 30 tests in the tissue analysis of grasses was 50 mg Zn·kg$^{-1}$ tissue with a range of 40-200 mg Zn·kg$^{-1}$ tissue. 'Merion' Kentucky bluegrass (Poa pratensis) tested 41 mg Zn·kg$^{-1}$ tissue at one location, red fescue (Festuca rubra) ranged from 43-50 mg Zn·kg$^{-1}$ tissue and bermudagrass (Cynodon dactylon) had Zn levels ranging from 20-50 mg Zn·kg$^{-1}$ tissue (Daniel and Freeborg, 1979). Madison (1971) found a range of 20-40 mg Zn·kg$^{-1}$ tissue in tall fescue (Festuca arundinacea).
B. Zn in Soils

The Zn content of the lithosphere is estimated to be about 80 mg Zn·kg⁻¹ with a common range of 10 to 300 mg Zn·kg⁻¹ total soil content (Swaine, 1955). As with other plant nutrients, total Zn concentrations correlate poorly to Zn availability. Hibbard (1940) found that surface soil contains more available Zn and total Zn than subsoil. He suggested that deposition of Zn by leaves and stems of plants caused the higher Zn concentration in the surface.

1. Diagnostic techniques

a. NH₄OAc-dithizone method  A correlation was shown between Zn deficiency and the amount of Zn extracted from soils by NH₄OAc and dithizone (Shaw and Dean, 1952). Massey (1957) reported a correlation between NH₄OAc-dithizone-extracted Zn and Zn uptake by corn plants but found a much higher correlation for a multiple regression where Zn uptake was calculated as a function of pH and extracted Zn. Tucker and Kurtz (1955) reported that the NH₄OAc-dithizone-extracted Zn was correlated with a bioassay method of estimating Zn availability. A correlation was shown between this method of extraction and the Zn availability as estimated by yield-of-Zn curves (Nearpass, 1956). The amount of Zn extracted by NH₄OAc-dithizone could be used to diagnose Zn deficiency in sweet corn (Brown and Krantz, 1960). This method seemed promising, particularly in areas of fairly uniform soil pH where the pH effect found by Massey (1957) would be small or absent (Berger and Pratt, 1963).

b. 0.1 N KCl method  The extraction of Zn using the 0.1 N HCl is a useful method in acid soils (Berger and Pratt, 1963). Wear and Sommers
(1948) used 0.1 N HCl-extracted Zn levels to determine whether applications of Zn were needed for corn grown on acid loams and sandy loams of Alabama. Correlations were found between the response of tung trees to Zn and the amount of 0.1 N HCl-extracted Zn (Barrows and Drosdoff, 1960). Also, Tucker and Kurtz (1955) found that 0.1 N HCl-extracted amounts of Zn that were significantly correlated with the bioassay method.

c. **DTPA method** Lindsay and Norvell (1969) reported use of DTPA (diethylenetriaminepentaacetic acid) as a soil test extractant for diagnosing Zn, Fe, Mn and Cu-deficient soils. Follett and Lindsay (1971) compared the decrease in DTPA-extractable Zn and other micronutrients following fertilization to what is generally known about the availability of each micronutrient cation. The results were in general agreement with residual responses reported from Zn and the other fertilizers which suggested that the DTPA soil test may be useful for monitoring the availability of micronutrient cations in fertilized and unfertilized soils (Follett and Lindsay, 1971).

2. **Factors affecting availability and movement of Zn**

The plant availability of Zn is affected by numerous soil and environmental factors: pH; adsorption on surfaces of clay; organic matter; carbonates and oxide minerals; complexation by organic matter; interactions with other nutrients; and climatic conditions (Tisdale et al., 1985).

a. **Soil pH** Deficiencies of Zn usually result when pH values are between 6 and 8 and decreases as the pH goes up or down (Thorne, 1957). Not all basic soils are Zn deficient because of mechanisms such as chelation of Zn by naturally occurring organic substances which may
compensate for the lower solubility of Zn at high pH (Tisdale et al., 1985).

Liming acid soils, particularly ones low in Zn, reduces Zn uptake. This effect is usually attributed to the effect that increasing pH has on lowering Zn solubility (Rogers and Wu, 1948; Winters and Parks, 1955). Another possibility is that some Zn could be adsorbed on the surface of freshly applied particles of liming agents such as CaCO₃ (Tisdale et al., 1985).

It is generally accepted that adsorption of Zn by clay minerals, various oxide minerals of aluminum, iron and magnesium, and other soil constituents is highly pH dependant with increases in pH leading to increases in adsorption (Tisdale et al., 1985). Adsorption of Zn by organic matter is also affected by pH. The amount of Zn complexed by humic acids increases with rising pH. Also, with increasing pH, the stability of Zn-organic complexes increase to a point after which the complexes break up and hydroxides form (Tisdale et al., 1985).

b. Interactions with phosphorus  Zinc deficiency often occurs on soils containing high levels of soluble and total phosphorus. Many of the results from such studies were confusing because the symptoms of Zn deficiency were not always accompanied by reductions in Zn concentrations in plant tops (Tisdale et al., 1985).

Loneragan (1975) and Christensen and Jackson (1981) found that when plants are deficient in Zn, their ability to regulate phosphorus accumulation is either lost or at least impaired. Therefore, phosphorus is absorbed by roots and transported to plant tops in large enough amounts to
become toxic. The resulting symptoms resemble Zn deficiency although Zn concentrations in plant tops are relatively unaffected.

C. Zn Deficiency

1. Effects on protein synthesis

The critical deficiency level for plants in general is below 15-20 mg Zn·kg⁻¹ dry leaf tissue (Marschner, 1986). The rate of protein synthesis and the protein content of Zn-deficient plants are drastically reduced (Marschner, 1986). In tomatoes and other plants, Zn deficiency causes large increases in amide and amino acid forms of protein precursors, particularly glutamine and asparagine (Davis, 1969).

At least three distinct mechanisms are responsible for the adverse effects of Zn deficiency on protein synthesis and protein content: Zn is a component of RNA polymerase; Zn is a component of ribosomes; and Zn deficiency increases the rate of RNase activity leading to increased RNA degradation (Johnson and Simmons, 1979).

2. Zn-phosphorus interactions

Large applications of phosphorus fertilizers to soils low in available Zn may induce Zn deficiency in plants and increase their Zn requirement (Loneragan et al., 1979). At least three factors may be involved: (a) "dilution" of Zn in plants by the phosphorus-induced growth increase, (b) inhibition of Zn uptake by the cations added with the phosphorus fertilizers, and (c) phosphorus-enhanced Zn adsorption in the soil to hydroxides and oxides of iron and aluminum and to CaCO₃ (Loneragan et al., 1979). Another possible phosphorus-Zn interaction in plants is inhibition of Zn translocation from roots to the shoot (Burleson and Page, 1967).
3. Deficiency symptoms

Under Zn-deficient conditions disintegration of ribosomes occurs, but reconstitution takes place when Zn is supplied to the plant (Marschner, 1986). Symptoms of Zn deficiency include yellowing and bronzing of stunted leaves and reduced growth as well as dark, thin desiccating leaves which whiten in advanced stages (Daniel and Freeborg, 1979).

D. Zn Toxicity

The most common symptom of Zn toxicity is the inhibition of root elongation which often leads to chlorosis of young leaves (Godbold et al., 1983; Powell et al., 1986; Woolhouse, 1983). In grasses, excess Zn causes small reddish patches between the veins (Madison, 1971).

1. Preventative/curative measures

Calcium increases the tolerance of plants to Zn (Baker, 1978). Inhibition of Zn uptake is involved, but tissue tolerance to high Zn levels also increases, presumably due to the role of Ca$^{++}$ ions in membrane stabilization and permeability, which is required for sequestering Zn in the vacuoles (Marschner, 1986; Wainwright and Woolhouse, 1977). Excess irrigation, and alkaline conditions reduce Zn availability. The surface soil accumulates a higher concentration of Zn than the lower levels since Zn is readily fixed. With excess phosphates, insoluble Zn compounds will precipitate from the soil (Daniel and Freeborg, 1979).

2. Causes of toxicity

A buildup in soil Zn levels could occur from excess application of a Zn containing fertilizer. Another less obvious source is Zn containing fungicides such as dithane, zineb and others (Daniel and Freeborg, 1979).
Cheaper grades of iron sulfate may also have Zn as an impurity from galvanized iron scrap used in its production (Madison, 1971).

3. Soil critical levels

Current standards for Zn levels within soils vary between soil testing laboratories. The soil lab at Iowa State University classifies a soil sample with 0.41 to 0.80 mg DTPA-extractable Zn·kg⁻¹ soil to be the adequate range for field crops and considers samples with Zn levels greater than 0.80 mg Zn·kg⁻¹ soil to have high amounts of available Zn (Eik, 1988). At Harris Labs in Lincoln, Nebraska, a soil sample with 1.6 to 2.0 mg DTPA-extractable Zn·kg⁻¹ soil is interpreted as the optimum level, and DTPA-extractable Zn levels above 2.0 mg Zn·kg⁻¹ soil are considered excessive (Frack, 1988). Neither lab has set a range of available Zn which it considers toxic to plants.

Soil analyses by Harris Labs for bentgrass golf greens from northern Iowa have shown excessive Zn levels. In separate sets of soil samples from 1984, 1987 and 1988 the average Zn levels were 53.2, 39.4, and 33.5 mg Zn·kg⁻¹ soil, respectively. Tissue analysis from six turf samples from these greens sent to Harris Labs in 1988, however, showed Zn levels ranging from 20 to 55 mg Zn·kg⁻¹ tissue which the lab considers the optimum range.

4. Examples in other species

In a sewage sludge study by Sanders et al. (1986), ryegrass (Lolium sp.) tolerated internal Zn levels ranging from 389 to 1190 mg Zn·kg⁻¹ tissue at pH levels from 7.2 to 5.8, respectively, without great yield reductions. Phytotoxicity, resulting in yield reduction, occurred at pH 4.7 and with the ryegrass tissue containing 2840 mg Zn·kg⁻¹ tissue.
Davis and Carlton-Smith (1984) found a total Zn concentration of 319 mg Zn·kg⁻¹ soil (with 159 mg Zn·kg⁻¹ soil being EDTA extractable) to be the upper critical soil level using 'Melle' perennial ryegrass (Lolium perenne) as an indicator crop.

Davis and Beckett (1977) concluded in a previous study that the minimum tissue concentration of Zn in ryegrass necessary to cause phytotoxicity was 221 mg Zn·kg⁻¹ tissue. This value was thought to be relatively independent of growing conditions.

The solubility of Zn is highly pH-dependant and decreases 100 fold for each unit increase in pH according to work done by W. L. Lindsay (1971) at Colorado State University. At low pH values some Zn may be present in a soil's exchange complex, but at higher pH values the Zn level in solution is so low that very little Zn is exchangeable as a cation (Lindsay, 1971).
III. MATERIALS AND METHODS

A. Initial Studies

Zinc toxicity studies were conducted in a climate controlled research greenhouse. The average light intensity across the greenhouse bench was 675 \( \mu \text{Mol} \cdot \text{sec}^{-1} \cdot \text{m}^{-2} \) with an average photoperiod of 14 hours. Air temperature was maintained at 25 ± 2°C.

The soil mix used in these studies contained sand, soil and Hypnum peat in an 8:1:1 ratio. The sand with a pH of 8.3 contained 180 g \( \text{CaCO}_3 \cdot \text{kg}^{-1} \) and fractions were as follows: 6% very coarse (2.0-1.0 mm diam.), 36% coarse (1.0-0.5 mm), 44% medium (0.5-0.25 mm), and 14% fine (0.25-0.1 mm). The soil was a Nicollet (fine loamy mixed mesic Aquic Hapuldoll) with a pH of 7.5 and 23 g organic matter \( \cdot \text{kg}^{-1} \) soil. The soil mix, itself, with a pH of 7.5, contained 17 g organic matter \( \cdot \text{kg}^{-1} \) soil, 28 mg potassium \( \cdot \text{kg}^{-1} \) soil, 5 mg phosphorus \( \cdot \text{kg}^{-1} \) soil, 1.3 g calcium \( \cdot \text{kg}^{-1} \) soil and 0.7 mg Zn \( \cdot \text{kg}^{-1} \) soil.

In the first study, initiated 15 Jan 1989, Agrostis palustris 'Penncross' was seeded in 103 cm\(^2\) pots each containing 500 g Zn-modified soil mix. The ZnSO\(_4\) was added at 0, 20, 40, 60, 80, 100, 120, 140, 160, 180 and 200 mg Zn \( \cdot \text{kg}^{-1} \) soil with three replications per treatment. Pots were seeded at 5.4 g \( \cdot \text{m}^{-2} \) and misted with distilled water until the seed had germinated. The treatments were arranged in a completely randomized design and watered to maintain surface moisture while minimizing leaching of nutrients through the bottom of the pots. Biweekly watering with modified Hoagland's solution (minus Zn) was begun after establishment (Hoagland and Aion, 1950). Clippings were removed after 12 weeks, dried at 80°C, dry
ashed, digested with 5 ml 1:1 HCl and analyzed on a Perkin-Elmer 403 Atomic Absorption Spectrophotometer. Soil samples, taken after 12 weeks, were analyzed for DTPA extractable Zn using the method of the Iowa State University Soils Testing Laboratory (Elk, 1985).

A second study was initiated 22 Mar 1989 using 'Penncross' creeping bentgrass sod. Treatments of ZnSO₄ at 0, 20, 40, 60, 80, 100, 120, 140, 160, 180, 200 mg Zn·kg⁻¹ soil in three replications each were mixed with 500 grams of the same soil mix and placed in 103 cm² pots. The sod was cut, washed to remove soil from the roots and placed on the surface of each pot. The pots were kept moist with distilled H₂O and with the biweekly, modified Hoagland's solution applications (Hoagland and Aion, 1950). Once the sod was established, biweekly Hoagland's solution applications were continued with distilled H₂O applied as needed to maintain the grass. Clippings were removed after 12 weeks, dried at 80°C, dry ashed, digested with 1:1 HCl, and analyzed on the Perkin-Elmer 403 Spectrophotometer for Zn concentration. Soil was sampled after 12 weeks and analyzed for DTPA-extractable Zn (Elk, 1985).

C. Third and Fourth Studies

Third and fourth studies were begun on 18 Nov 1989 and 08 Dec 1989, respectively, using 'Penncross' creeping bentgrass sod. Completely randomized treatments of ZnSO₄ at 0, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1500, and 2000 mg Zn·kg⁻¹ soil in four replications were mixed with 500 grams soil mix, placed in 103 cm² pots, moistened with distilled water and allowed to equilibrate for one month. The sod was cut, washed to remove soil from the roots and placed on the surface of each pot for both
studies. The pots were kept moist with distilled H₂O and with the biweekly modified Hoagland's solution applications (Hoagland and Aínón, 1950). Once the sod was established, biweekly Hoagland's solution applications were continued with distilled H₂O applied as needed to maintain the grass.

Clippings were removed from each pot of grass after 12 weeks. Small quantities of the fresh tissue from each pot were collected for chlorophyll (Chl) analysis. The remainder was dried at 80°C, dry ashed, digested with 1:1 HCl, and analyzed on the Perkin-Elmer 403 Spectrophotometer for the Zn concentration.

Chlorophyll content of the subsamples was determined by a modification of the method by Knudson et al. (1977). Leaves from each sample were cut into small pieces and placed into test tubes with 10 ml of 95% ethanol and extracted for Chl in the dark for 24 hours. The extractions were repeated three times and combined (30 ml total volume). Chlorophyll absorbencies were measured spectrophotometrically at 665 and 649 nm, and appropriate calculations made to determine total Chl. Chl concentrations were reported as μg Chl·mg⁻¹ dry leaf weight.

Soil was sampled after 12 weeks and analyzed for DTPA-extractable Zn (Eik, 1985). Data from these studies were combined for analysis and presentation since they were replicates in time.

D. Fifth and Sixth Studies

Fifth and sixth studies were begun on 9 Mar 1990 and 23 Mar 1990, respectively, using 'Penncross', 'Penneagle', 'Emerald', 'Cobra' and 'Prominent' creeping bentgrass sod established from seed in the ISU Horticulture greenhouses. Completely randomized treatments of ZnSO₄ at 0,
500, 1000, 2000 and 4000 mg Zn·kg⁻¹ soil in four replications per study were mixed with 500 grams soil mix, placed in 103 cm² pots, moistened with distilled water and allowed to equilibrate for three weeks. The sod was cut, washed to remove soil from the roots and placed on the surface of each pot for both studies. The pots were kept moist with distilled H₂O and with the biweekly modified Hoagland's solution applications (Hoagland and Amin, 1950). Once the sod was established, biweekly Hoagland's solution applications were continued with distilled H₂O applied as needed to maintain the grass.

Clippings were removed from each pot of grass after 10 weeks. Small quantities of the fresh tissue from each pot were collected for chlorophyll (Chl) analysis. The remainder was dried at 80° C, dry ashed, digested with 1:1 HCl, and analyzed on the Perkin-Elmer 403 Spectrophotometer for the Zn concentration.

Chlorophyll content of the subsamples was determined by a modification of the method by Knudson et al. (1977). Leaves from each sample were cut into small pieces and placed into test tubes with 10 ml of 95% ethanol and extracted for Chl in the dark for 24 hours. The extractions were repeated three times and combined (30 ml total volume). Chlorophyll absorbencies were measured spectrophotometrically at 665 and 649 nm, and appropriate calculations made to determine total Chl. Chlorophyll concentrations were determined as µg Chl·mg⁻¹ dry leaf weight.

Soil was sampled after 10 weeks and analyzed for DTPA-extractable Zn (Eik, 1985). Data from these studies were combined for analysis and presentation since they were replicates in time.
IV. RESULTS

A. Initial Studies

Similar trends were observed in the first two studies with the 'Penncross' creeping bentgrass and their results were combined for analysis and presentation (Fig. 1). There was a linear increase in Zn tissue concentration with increasing treatment levels. The DTPA-extractable Zn concentrations of the soil samples taken after 12 weeks also followed a linear trend. Soil concentration means ranged from 15 mg DTPA-extractable Zn·kg⁻¹ soil in the control to 72 mg DTPA-extractable Zn·kg⁻¹ soil in the 200 mg Zn·kg⁻¹ soil treatment (Fig. 1). The mean Zn concentration in the tissue sampled after 12 weeks ranged from 37 mg Zn·kg⁻¹ tissue in the control plants to 102 mg Zn·kg⁻¹ tissue in plants growing in soil treated with 200 mg Zn·kg⁻¹ soil (Fig. 1). No toxicity was observed on the plants at any of the Zn treatment levels in the initial studies.

The initial studies provided useful information for subsequent studies. Increasing Zn application lead to increasing tissue Zn concentrations and soil extractable concentrations. Applications of Zn at 200 mg Zn·kg⁻¹ soil mix did not elevate tissue Zn concentrations above the range of 40-200 mg Zn·kg⁻¹ tissue measured by Boehle and Lindsay (1969) in turf stands. It was obvious that higher levels of applied Zn would be needed to have a phytotoxic effect on 'Penncross' creeping bentgrass.

B. Third and Fourth Studies

Soil samples from the combined data of the third and fourth studies had mean DTPA-extractable Zn levels ranging from 7 mg Zn·kg⁻¹ soil in control pots to 534 mg Zn·kg⁻¹ soil in pots treated with 2000 mg Zn·kg⁻¹
soil (Fig. 2). The tissue samples from the studies had mean Zn levels ranging from 110 mg Zn·kg⁻¹ tissue for the control units to 1375 mg Zn·kg⁻¹ tissue for the units treated with 2000 mg Zn·kg⁻¹ soil (Fig. 2). Some slight but inconsistent chlorosis was seen starting about 6 weeks after sod establishment on the grass in pots treated with 900, 1000, 1500 and 2000 mg Zn·kg⁻¹ soil. Differences between total Chl content of grass treatments were not significant (Table I).

Application of Zn at 2000 mg Zn·kg⁻¹ soil increased internal Zn levels beyond the range tolerated by sewage sludge treated ryegrass (Sanders et al., 1986). The mean DTPA-extractable Zn concentration of 534 mg Zn·kg⁻¹ soil was well above the upper critical level for 'Melle' perennial ryegrass (Lolium perenne) as established by Davis and Carlton-Smith (1984). 'Penncross' creeping bentgrass may have some degree of tolerance to high internal Zn levels like the metal-tolerant clones of Agrostis tenuis Sibth., Agrostis stolonifera L. and other species (Gregory and Bradshaw, 1965). The next logical step was to treat 'Penncross' and other varieties of creeping bentgrass with higher levels of Zn.

C. Fifth and Sixth Studies

Soil samples from the combined data of the final studies had mean DTPA-extractable Zn levels ranging from 0.6 mg Zn·kg⁻¹ soil in control pots to 652 mg Zn·kg⁻¹ soil in the 4000 mg Zn·kg⁻¹ soil treated pots (Fig. 3). Differences among varieties in DTPA-extractable Zn levels were not significant (Table II). The tissue samples from the final studies had mean Zn levels ranging from 50 to 135 mg Zn·kg⁻¹ tissue for the control units to 976 to 1500 mg Zn·kg⁻¹ tissue for the 4000 mg Zn·kg⁻¹ soil treated units.
depending upon the variety (Fig. 3). Differences between varieties in tissue Zn concentrations were significant (Table II). Inconsistent yellowing was seen starting about 6 weeks after sod application on some of the replicates of all grass varieties treated with 2000 and 4000 mg Zn·kg⁻¹ soil. However, differences between total Chl content of grass treatments were not significant (Table II).

Increasing the maximum treatment level from 2000 to 4000 mg Zn·kg⁻¹ soil changed the regression line from a linear to a quadratic relationship (Fig. 3). The DTPA-extractable Zn concentrations in the 4000 mg Zn·kg⁻¹ soil treated pots was less than expected in relation to the 2000 mg Zn·kg⁻¹ soil treated pots. There was a linear increase in mean tissue Zn concentrations for the varieties 'Penncross', 'Penneagle', and 'Emerald', but the mean tissue Zn concentrations for the varieties 'Cobra' and 'Prominent' increased quadratically like the DTPA-extractable Zn concentrations (Fig. 3). The root:shoot ratios of Zn could be higher in the latter two varieties than in the former three varieties as occurred in studies comparing Zn distribution between tolerant and intolerant clones of grass species (Peterson, 1969). However, no differences were apparent between varieties in the ability to tolerate these high levels of zinc as measured by Chl levels (Table II).

The studies were not designed to include measurements of rooting quality or leaf clipping weights. These data may have shown greater differences between treatments since zinc toxicity commonly inhibits root elongation which often leads to chlorosis of young leaves (Godbold et al., 1983; Powell et al., 1986; Woolhouse, 1983).
Table I. Analysis of variance for the DTPA-ext. Zn levels (soil), tissue Zn concentrations (tissue) and total Chl concentrations (tcl) from combined data of studies 3 and 4

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**Significant at 0.01 level.

ns=no significance.
Table II. Analysis of variance for the DTPA-ext. Zn levels (soil), tissue Zn concentrations (tissue) and total Chl concentrations (tcl) from combined data of studies 5 and 6

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*Significant at 0.05 level.
**Significant at 0.01 level.
ns=no significance.
Figure 1. Means of the combined data from studies 1 and 2 of DTPA-extractable Zn concentrations (mg Zn·kg⁻¹ soil) and Zn concentrations of the grass tissue (mg Zn·kg⁻¹ tissue) as related to the Zn application level (mg Zn·kg⁻¹ soil)
Figure 1

- **DTPA-ext. Zn (soil)**
  - Regression Line
  - $r^2 = 0.59$
  - $\hat{Y} = 6.28 + 0.3 \times X$

- **Tissue Zn Conc.**
  - Regression Line
  - $r^2 = 0.38$
  - $\hat{Y} = 48.4 + 0.3 \times X$
Figure 2. Means of the combined data for studies 3 and 4 of DTPA-extractable Zn concentrations (mg Zn·kg⁻¹ soil) and Zn concentrations of the grass tissue (mg Zn·kg⁻¹ tissue) 12 weeks after treatment application as related to the Zn application level (mg Zn·kg⁻¹ soil)
Figure 2

- DTPA-ext. Zn (soil)
- Regression Line

\[
r^2 = 0.77
\]
\[
\hat{y} = -12.7 + 0.28 \times \]

- Tissue Zn Conc.
- Regression Line

\[
r^2 = 0.73
\]
\[
\hat{y} = 67.5 + 0.65 \times \]

mg applied Zn/kg soil vs. mg ext. Zn/kg soil

mg Zn/kg tissue vs. mg applied Zn/kg soil
Figure 3. Means of the combined data from the studies 5 and 6 of DTPA-extractable Zn concentrations (mg Zn·kg⁻¹ soil) and Zn concentrations of the grass tissue (mg Zn·kg⁻¹ tissue) 10 weeks after treatment application as related to the Zn application level (mg Zn·kg⁻¹ soil)
Figure 5

\[ r^2 = 0.91 \]
\[ \hat{Y} = 0.33 \times X - 0.00004 \times X^2 \]

Figure 3
Table III. Regression analysis by variety for tissue Zn concentrations from the combined data of studies 5 and 6

<table>
<thead>
<tr>
<th>Variety</th>
<th>Regression equation</th>
<th>$r^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penneagle</td>
<td>$\hat{Y} = 87 + 0.32 \ (X)$</td>
<td>0.65</td>
</tr>
<tr>
<td>Penncross</td>
<td>$\hat{Y} = 168 + 0.29 \ (X)$</td>
<td>0.52</td>
</tr>
<tr>
<td>Cobra</td>
<td>$\hat{Y} = 29 + 0.44 \ (X) - 0.00005 \ (X^2)$</td>
<td>0.81</td>
</tr>
<tr>
<td>Prominent</td>
<td>$\hat{Y} = 48 + 0.35 \ (X) - 0.00003 \ (X^2)$</td>
<td>0.90</td>
</tr>
<tr>
<td>Emerald</td>
<td>$\hat{Y} = 34 + 0.37 \ (X)$</td>
<td>0.82</td>
</tr>
</tbody>
</table>
V. SUMMARY AND CONCLUSIONS

Levels of Zn up to 2000 mg Zn·kg\(^{-1}\) soil were applied to 'Penncross' creeping bentgrass in studies 3 and 4. In studies 5 and 6 up to 4000 mg Zn·kg\(^{-1}\) soil was applied to 'Penncross', 'Penneagle', 'Prominent', 'Emerald', and 'Cobra' creeping bentgrass. These treatments increased the mean DTPA-extractable Zn soil concentration from 0.6 mg Zn·kg\(^{-1}\) soil of the control pots to 652 mg Zn·kg\(^{-1}\) soil in the 4000 mg Zn·kg\(^{-1}\) soil treated pots. The mean tissue Zn concentrations increased from a low of 50 mg Zn·kg\(^{-1}\) tissue for grass on control pots to a high of 1500 mg Zn·kg\(^{-1}\) tissue for grass treated with 4000 mg Zn·kg\(^{-1}\) soil. No consistent deleterious effects were seen in these studies on any of these creeping bentgrass varieties.

The stress of heavy foot traffic, ball and cleat marks, frequent mowing, heavy fertilization and irrigation, diseases, and temperature extremes are some of the additional factors which may affect the ability of creeping bentgrass on golf greens to tolerate high levels of available Zn. However, it is unlikely that DTPA-extractable Zn concentrations in the range of 20 to 60 mg Zn·kg\(^{-1}\) soil that have been identified as excessive amounts of available Zn by some soil testing labs, pose a risk of Zn toxicity to creeping bentgrass on high sand golf greens.

Plants generally require less than 2.0 mg DTPA-extractable Zn·kg\(^{-1}\) soil to maintain adequate tissue Zn concentrations. Soil Zn concentrations in this range are generally listed as the optimum level on soil test reports (Frack, 1988; Eik, 1988). The terms to describe greater levels vary from 'high' to 'excessive'. The term excessive can be misleading and
confusing. It may have been used due to concerns about the effects of Zn on grazing animals. This concern does not apply to turfgrass since clippings are not generally fed to livestock.

While the Zn requirement of creeping bentgrass is quite low, the term excessive implies that the Zn concentration in the soil is very high and perhaps hazardous. Based on the results of these studies, Zn levels would have to be well above the 20 to 60 mg Zn·kg⁻¹ soil, presently labeled as 'excessive Zn', to damage creeping bentgrass.
VI. BIBLIOGRAPHY


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Frack, S. 1988. Personal communication. Harris Laboratories, Inc. Lincoln, NE.


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