MANGANESE TOXICITY TO PENNCROSS BENTGRASS

by
DAVID EUGENE CREWS

DEPARTMENT OF CROP SCIENCE

RALEIGH
1974

ABSTRACT

CREWS, DAVID EUGENE, Manganese Toxicity to Penncross Bentgrass. (Under the direction of WILLIAM B. GILBERT).

The use of river terrace soils in building bentgrass golf greens in the South has rapidly increased. Some of these soils have accumulated Mn to levels that may be toxic to *Agrostis palustris* var. Penncross, the most commonly used bentgrass in the South. The purpose of this investigation was to determine the toxic levels of Mn to Penncross bentgrass, and to study the effect of lime and P on the high Mn levels in golf green soil mixes made from river terrace soils.

In nutrient solution experiments, the toxic concentration of Mn in the plant parts exceeded 2000 ppm in the tops and 7000 ppm in the roots. Toxic symptoms in the tops and roots of plants clipped weekly to 1.3 cm occurred at 8.0 and 16.0 ppm solution Mn respectively. Unclipped plants tolerated four times the concentration of solution Mn tolerated by clipped plants. Increasing the solution Mn level (a) decreased top yield in clipped plants, (b) had no effect on top yield in unclipped plants, and (c) increased the root weight in both clipped and unclipped plants.

In soil experiments, application of lime to the soil mixes increased Mn extracted with .05 *N* HCl plus .025 *N* H_2SO_4 (acid-extractable Mn), but decreased Mn extracted with NH_4OAc (exchangeable Mn). Phosphorous had little effect on either extractant method. Plant Mn concentrations were reduced significantly by applications of lime and P, and correlated better with exchangeable Mn than with acid

extractable Mn. An increase in top Mn over all lime and P treatments occurred between harvests of bentgrass grown in a soil mix high in Mn, but did not approach the determined toxic level.

MANGANESE TOXICITY TO PENNCROSS BENTGRASS

by

DAVID EUGENE CREWS

A thesis submitted to the Graduate Faculty of
North Carolina State University at Raleigh
in partial fulfillment of the
requirements for the Degree of
Master of Science

DEPARTMENT OF CROP SCIENCE

RALEIGH

1 9 7 4

APPROVED BY:

Chairman of Advisory Committee

BIOGRAPHY

The author was born on March 21, 1949, to Mr. and Mrs. Francis Hylton Crews. He was raised in Winston-Salem, North Carolina, where he was graduated from Parkland High School in 1967. In the fall of 1967 he entered North Carolina State University where he was a member of Alpha Zeta National Honorary Fraternity, Agronomy Club, and the University varsity golf team. He was graduated in 1971 after obtaining his Bachelor's degree in Agronomy. In the fall of 1971, he entered the Crop Science Department of North Carolina State University to obtain his Master's degree.

ACKNOWLEDGMENTS

The author wishes to express his sincere gratitude to Dr. W. B. Gilbert, Chairman of his Advisory Committee, for his guidance and assistance throughout the course of this investigation. Additional appreciation is extended to the other members of the Advisory Committee, Dr. D. S. Chamblee, Dr. F. R. Cox, and Dr. D. E. Moreland, for their help in preparing this manuscript. Special thanks are extended to Dr. J. B. Sartain for his assistance in laboratory procedures and statistical analyses.

Grateful appreciation is extended to the United States Golf Association for providing the funding which made this investigation possible.

Finally, the author would like to thank his parents, Mr. and Mrs. Francis Hylton Crews for their time, patience, and encouragement throughout this study.

TABLE OF CONTENTS

	Page
LIST OF TABLES	v
LIST OF FIGURES	vii
INTRODUCTION	1
REVIEW OF LITERATURE	4
Soil Manganese Availability	4
Soil Manganese Toxicity	7
Soil Manganese Extractions	8
Plant Manganese	9
Plant Manganese Tolerance	11
MATERIALS AND METHODS	14
Experiment I Nutrient Solution Study	15
Experiment II Nutrient Solution Study	16
Experiment III Soil Study	16
Experiment IV Soil Study	18
RESULTS AND DISCUSSION	20
Experiment I	20
Experiment II	27
Experiment III	45
Experiment IV	52
SUMMARY	61
LIST OF REFERENCES	63

LIST OF TABLES

Page

1. Effect of nutrient solution Mn levels on top dry weight, root dry weight, and the top/root ratio of Penncross bentgrass 21

2. Effect of nutrient solution Mn levels on the Mn concentration and total Mn uptake in the tops and roots of Penncross bentgrass 25

3. Effect of nutrient solution Mn levels on the leaf width of Penncross bentgrass 29

4. Effect of nutrient solution Mn levels on the top dry weight of Penncross bentgrass at three harvest dates . . 30

5. Effect of nutrient solution Mn levels on the concentration of Mn in the dry plant tops of Penncross bentgrass at three harvest dates 31

6. Effect of nutrient solution Mn levels on the total Mn in the dry plant tops of Penncross bentgrass at three harvest dates. 32

7. Effect of nutrient solution Mn levels on root weight, root Mn concentration, and total root Mn in Penncross bentgrass 33

8. Effect of nutrient solution Mn levels on the concentrations of Fe, Ca, Mg, and Zn in the dry plant tops of Penncross bentgrass 37

9. Effect of nutrient solution Mn levels on the total Fe, Ca, Mg, and Zn in the dry plant tops of Penncross bentgrass 38

10. Effect of nutrient solution Mn levels on the concentrations of Fe, Ca, Mg, and Zn in the dry plant roots of Penncross bentgrass 39

11. Effect of nutrient solution Mn levels on the total Fe, Ca, Mg, and Zn in the dry roots of Penncross bentgrass 40

12. Effect of nutrient solution Mn levels on the top/root ratio of Fe, Ca, Mg, and Zn in Penncross bentgrass . . 41

13. Regression models for top yield, root yield, and top/root ratios. 44

LIST OF TABLES (Continued)

Page

14. Effect of applications of lime and phosphorous on some chemical properties of soil I 46

15. Effect of applications of lime and phosphorous on some chemical properties of soil II. 47

16. Effect of applications of lime and phosphorous on some chemical properties of soil III 48

17. Effect of applications of lime and P on the amount of exchangeable and extractable Mn in soil III. . . . 54

18. Effect of applications of lime and P on the top Mn concentration of Penncross bentgrass grown on soil III at two harvest dates 58

LIST OF FIGURES

Page

1. Effect of nutrient solution Mn levels on the top/root uptake ratio of Penncross bentgrass 23

2. Effect of nutrient solution Mn levels on the top/root Mn concentration ratio of Penncross bentgrass. 24

3. Effect of nutrient solution Mn levels on the top/root ratio of Penncross bentgrass clipped weekly to 1.3 cm 35

4. Effect of nutrient solution Mn levels on the top/root Mn uptake ratio of Penncross bentgrass clipped weekly to 1.3 cm 36

5. Effect of lime and P on the mean acid-extractable Mn level of three golf green soils 49

6. Effect of lime and P on the mean top yield of Penncross bentgrass grown on three soils. 53

7. Effect of lime and P on the amount of exchangeable Mn in soil III 55

8. Relation between Mn concentration in tops of Penncross bentgrass and NH_4OAc extractable Mn in soil III 59

INTRODUCTION

In recent years there has been a rapid increase in the use of bentgrass for golf greens in the southeastern United States. Previously, bentgrass golf greens have been located in the northern states where the climate was more conducive for the growth of this cool season grass. Now, however, due to better adapted grass varieties, improved greens construction, more efficient fungicides, and an overall desire by the golfing public for finer putting surfaces, bentgrass golf greens are assuming a more dominant role in the southeast.

In building bentgrass golf greens one must consider the physical and chemical properties of the soils used. Once the green is built and in use these properties are difficult to alter. Soil structure, texture, and drainage are all very important when choosing the top-soil mix to use. The mix should be free of compounds detrimental to the normal growth of the grass. In the southeast there has been a trend toward using river terrace soils as a part of the top-soil mix for bentgrass golf greens because of their desirable structure and texture. Before these soils are used in greens construction however, they should be analyzed for possible toxic accumulations of any elements. Pedogenic processes involved in river terrace soil formation are conducive to accumulations of elements such as manganese (Mn), iron (Fe), and aluminum (Al). Buol (1974) said Mn accumulation in terrace soils is a two step process. First, Mn goes into solution, a process caused by reducing conditions in soils with impermeable subsoils in upland positions.

In North Carolina, parent materials in the "Triassic and Slate" belts are typically high in Mn, Fe, and Al. Second, the manganous Mn is carried downslope and deposited in better oxidized terrace soils. Tiller (1963) found up to 95% of the Mn in soils in reducing enviroments could be lost by lateral water movement. Pitty (1971) reported the floodplain soil of the Vikhra River contained five times more Mn and five to eight times more Fe than the soil of the adjacent watershed.

In North Carolina the Mn levels in the river terrace soils may not be high enough to cause severe toxicity symptoms immediately after greens construction, but they may be high enough to have a detrimental effect on the grass over an extended period of time. In golf greens a reducing environment is prevalent, which would further increase Mn availability in the soil. It is therefore important to determine before greens construction if the Mn in the soil is at or near the toxic level to the grass. After greens construction, a satisfactory correction of the problem would be difficult.

Generally, soils on which Mn toxicity occurs are either acidic and/or waterlogged. Correction of the problem is usually accomplished through drainage and/or liming to a higher pH. The problem is not so easily solved when working with a golf green. Some features of golf greens and golf greens maintenance are conducive to high levels of available soil Mn. The top 1.3 to 3.8 cm of soil and thatch tend to be more acid than the rest of the soil. Liming this layer to a higher pH could create deficiencies of other essential nutrients. Heavy use of water cuts down on soil aeration, increases soil compaction, and facilitates the reduction of manganic oxides to plant available Mn^{+2}. Use of pesticides containing Mn or use of acid forming fertilizers may also result in increased available soil Mn.

A major objective of this study was to determine the toxic level of Mn to Penncross bentgrass. The effect of Mn on other essential elements was also examined.

A second objective was to determine the effect of lime and phosphorous (P) on the availability of Mn in three golf green mixes made from river terrace soils.

REVIEW OF LITERATURE

Soil Manganese Availability

To evaluate the availability of soil manganese (Mn) in relationship to plant growth, the various forms of Mn in the soil must be considered. Tisdale and Nelson (1966) suggested that soil Mn existed in three valence states: Mn^{+2}, Mn^{+3}, and Mn^{+4}. The Mn^{+2} was present in the soil as an adsorbed cation or in the soil solution as hydroxides, carbonates, and silicates. This was the Mn available for plant growth. The Mn^{+3} existed as a highly reactive oxide Mn_2O_3, and the Mn^{+4} existed as an inert oxide MnO_2. Leeper (1947) separated soil Mn into two fractions: 1) exchangeable, nonexchangeable, and water soluble; and 2) insoluble higher oxides. He suggested that these forms existed in dynamic equilibrium. Geering et al. (1969) described soil Mn similar to Leeper (1947), but he included an organically bound Mn not available to plants.

Availability of soil Mn to plants is influenced by a number of factors. These include soil pH, redox potential, and organic matter. Tisdale and Nelson (1966) found a direct relationship between soil pH and Mn extracted by a neutral salt. As the pH was increased by liming a black sandy loam soil from pH 5.0 to 7.0, extractable Mn decreased from 1.4 to 0.2 ppm. Robie and Waldbaum (1972) found that as the pH of a soil increased, the various Mn ions and minerals decreased in solubility. The predominant ion in solution was Mn^{+2}, while MnO_2 was the most stable mineral in solution.

Several theories have been proposed to explain the changes in availability of soil Mn with increasing soil pH. Mulder and

Gerretsen (1952) stated that the major portion of available Mn in a soil of pH less than 5.5 may exist in the water soluble and/or exchangeable form. As the pH increased because of liming, the Mn^{+2} was converted to less available manganic oxides. Heintze (1946) found that one week after liming a clay loam soil from pH 4.5 to 7.9, 80% of the extractable Mn had been converted to insoluble manganic oxides. On neutral or alkaline soils it has proven very inefficient to broadcast applications of Mn fertilizers because of the rapid oxidation of the Mn (Lucas and Knezek, 1972). Instead, farmers make a band application of Mn in combination with an acid fertilizer. In this manner, Mn oxidation is reduced. The acid solution diffusing from the fertilizer aids in increasing the available Mn level.

Other investigators have reported that Mn is tied up by organic matter in the soil when the soil pH is increased. Page (1962) stated that available Mn levels were not lowered by Mn^{+2} being converted to insoluble higher oxides. Instead, the Mn^{+2} was complexed with organic matter in the soil. Heintze and Mann (1949) found that insoluble Mn may be complexed with organic matter in organic soils that were neutral or alkaline. Mulder and Gerretsen (1952) reported that Mn deficiency on a soil with a pH of 6.0 or more increased with organic matter content.

Redox potential of a soil is often related to the levels of available soil Mn. Tisdale and Nelson (1966) stated that the oxidation potential for conversion of Mn^{+2} to MnO_2 was a linear function of pH values from 3.8 to 8.0. They did not conclude whether pH or oxidation status of the soil was more closely related to Mn availability. Redox potential is affected by soil moisture, aeration, and bacterial respiration (Corey and Schulte, 1972). It affects the availability of elements that can exist in more than one oxidation state. In a well-oxidized soil, Mn exists as the +4 ion in MnO_2. Under reducing conditions, however, such as a waterlogged soil where air movement and aerobic respiration are at a minimum, the Mn oxide is converted to the Mn^{+2} ion. This is shown in the following equation:

$$MnO_2 + 4H^+ + 2e^- \rightleftharpoons Mn^{+2} + H_2O$$

Investigators have shown that flooding of fields could temporarily correct Mn deficiency symptoms (Mulder and Gerretsen, 1952) and that a combination of flooding, organic matter, and high temperature could increase Mn in solution to 46.0 ppm in some soils (Meek *et al.* 1968). Graven *et al.* (1965) found in greenhouse studies that, regardless of pH, flooding increased the Mn content of alfalfa. In the absence of easily decomposible organic matter, Mn mobilization by flooding was faster at pH 7.3 than 4.7. In field studies it was found that 72 hours of flooding increased the Mn concentration of alfalfa from 426 to 7686 ppm under acid conditions, and from 99 to 1290 ppm when 2500 ppm of $CaCO_3$ was added to the soil.

It is well established that organic matter affects soil Mn availability. Heintze and Mann (1947) found that organic acid-Mn complexes existed and were soluble over a wide range of pH's. They suggested that such a complex may be important in maintaining adequate levels of available soil Mn. Hemstock and Low (1953) reported that Mn retention in soils absent of oxygen and biological activity may be in the form of a chelated complex. Using a Maumee sandy loam, they found that the soil contained nonexchangeable Mn that could be extracted with copper salts. The Mn came from organic combinations and not manganic oxides. They suggested a simple Mn-Cu exchange to explain the release of the chelated Mn. Sanchez and Kamprath (1959) found that additions of peat to an acid, low organic matter soil increased the exchangeable Mn content. Additions of peat to a limed soil decreased the acid-extractable Mn.

Soil Manganese Toxicity

Manganese toxicity is usually associated with soils having pH values below 5.5, or soils that are poorly aerated or waterlogged. Berger and Gerloff (1947) reported stem necrosis in potatoes grown on Wisconsin soils having pH values less than 5.5. He found this was caused by excess Mn in the stems. On an Onamia fine sandy loam and a Vilas sand, both with a pH of 4.8, he found water soluble Mn levels of 2.2 and 4.3 ppm, respectively. When the two soils were limed to pH values of 6.8 and 5.9, the water soluble Mn levels dropped to 0.0 and 0.1 ppm. There were indications that water soluble Mn was a better indicator of Mn contributing to toxicity than exchangeable Mn. When potatoes were grown in a solution culture, 3.0 ppm Mn gave toxic symptoms, but 1.0 ppm Mn did not. Cheng and Ouelette (1968) reported that potatoes grown on a soil having a pH of 4.5 and an exchangeable Mn level of 12.0 ppm Mn showed toxic symptoms. They reported 5.0 ppm Mn in a sand culture to be toxic to potatoes. Schnehl *et al.* (1950) reported Mn toxicity in alfalfa grown on very acid soil, but indicated that yield was not reduced until toxic symptoms became very severe. Liming the soil reduced both the available Mn and the toxic symptoms on the plant.

Flooding of soils can result in the reduction of Mn^{+4} to Mn^{+2} and raise available plant Mn to toxic levels (Mulder and Gerretsen, 1952; Meek et al., 1968; and Bradfield et al. 1934). Investigators relate this to changes in the redox potential of the soil.

Timonin (1946) showed that fumigation of soils with chloropicrin, cyanogas, or formaldehyde reduced the population of soil bacteria able to oxidize Mn. Crops that had previously shown Mn deficiencies on these soils now showed no deficiency symptoms. Smith (1963) found in field studies that fumigation with methyl bromide (CH_3Br) significantly increased the amount of ammonium acetate (NH_4OAc) extractable Mn thirty months after fumigation. He concluded the persistence was because of suppression of Mn oxidizing organisms. If soils high in total Mn were fumigated, Mn toxicity may be likely in crops grown on these soils until Mn levels were reduced to normal by oxidation and micro-organisms.

Soil Manganese Extractions

There seems to be disagreement in the literature as to which soil Mn extractant best correlates with plant growth. This is because Mn exists in the soil in different forms, and each has its own availability. Among the more successful extractants are water, 1N NH_4OAc, 0.2% hydroquinone, and weak acids. Manganese extracted with 1N NH_4OAc is often referrred to as extractable Mn, while Mn extracted with 0.2% hydroquinone is often called easily reducible Mn. Morris (1948) found that water soluble Mn was a better indicator of Mn toxicity in lespedeza than extractable Mn. Other investigators have found this to be true in potatoes (Berger and Gerloff, 1947), tobacco (Bortner, 1935), and cotton (Adams and Wear, 1957).

Hale and Heintze (1946) reported that soils on which Mn injury had occurred were characterized by a low pH (below 5.5) and a high NH_4OAc extractable Mn level. Rich (1956) found that NH_4OAc extracted Mn correlated better with leaf Mn concentrations in peanuts than that extracted with 0.2% hydroquinone. Fergus (1954) stated that soils below pH 5.0 had a potential for causing Mn toxicity if the easily reducible Mn level was high. He concluded that extractable Mn was not reliable in predicting toxicity. Hoff and Mederski (1958) reported that of nine methods tested for estimating available Mn, $NH_4H_2PO_4$, alcoholic hydroquinone, and H_3PO_4 gave the highest correlation coefficients. Hoyt and Nyborg (1971) found that a 16-hour extraction with .01$\underline{M}$ $CaCl_2$ was the best estimator of available Mn. They concluded this by extracting Mn from forty soils and comparing these amounts with the Mn concentrations in the leaves of barley, rape and alfalfa plants grown on these soils. Extractions with 1$\underline{N}$ NH_4OAc, 0.1$\underline{N}$ H_3PO_4, and 0.2% hydroquinone gave only fair to poor results.

Cox (1968) used an acid extraction (1:4 soil/.05$\underline{N}$ HCl plus .025$\underline{N}$ H_2SO_4) to correlate the response of soybeans to Mn fertilization. He found the test useful over a pH range of 5.2 to 7.1. Hames and Berger (1960) reported that Mn extracted with 0.1$\underline{N}$ H_3PO_4 correlated better with Mn uptake in oats than extractions with 1.5$\underline{M}$ $NH_4H_2PO_4$ or 1.0$\underline{N}$ H_3PO_4.

Plant Manganese

It is well established that Mn plays a role in photosynthesis. Additionally there are some indications that Mn affects the structure of chloroplasts. Homann (1967) found that higher plants responded to

Mn deficiency by reducing the number of chloroplasts per cell, or by forming disorganized chloroplasts with low chlorophyll content. Anderson and Pyliotis (1969) reported a change in the chlorophyll b/chlorophyll a ratio in spinach chloroplasts deficient in Mn. Teicher-Zallen (1969) suggested that Mn played a role in chloroplast structure.

The principal function of Mn is associated with Photosystem II of photosynthesis, with little or no function in Photosystem I. Cheniae and Martin (1970), Heath and Hind (1969) and Itoh et al. (1969) reported that Mn functioned in Photosystem II on the oxidizing side, while Gavalas and Clark (1971) found Mn to function on the reducing side of Photosystem II. All agreed Mn played a role in the evolution of oxygen.

Some observers related Mn toxicity to Mn-Fe interactions. Weinstein and Robbins (1955) found Fe deficiency symptoms with high Mn concentrations in sunflowers. High Mn levels also resulted in low protein content. They suggested that the Fe deficiency was caused by competition between Mn and Fe for sites on Fe containing enzymes. Shive (1941) and Somers and Shive (1944) reported that high Mn levels in tissues were associated with low Fe tissue concentrations. They concluded that oxidation of ferrous to ferric ions took place, thereby inactivating the Fe. Increasing available Fe to St. Augustinegrass (Snyder and Gascho, 1973) and burley tobacco (Hiatt and Ragland, 1963) has been shown to overcome Mn toxicity symptoms in these plants.

Only one enzyme, pyruvate carboxylase, has been shown to be Mn activated (Scrutton et al., 1966). Anderson and Evans (1956) reported that toxic levels of Mn in some plants resulted in a two- to threefold increase in isocitric dehydrogenase and malic enzyme. Indolacetic acid oxidase activity increased and indolacetic acid oxidase inhibitor decreased in cotton when Mn was applied at a toxic level (81 ppm) in a sand culture (Morgan, 1966). Toxic symptoms were related to an auxin deficiency and high Mn levels. Stonier _et al._ (1968) found that toxic levels of Mn catalyzed the oxidation of auxin protectors in Japanese honeysuckle, and accelerated the oxidation of indolacetic acid by peroxidase.

Plant Manganese Tolerance

Plant species differ in their tolerance to excess Mn. Williams and Vlamis (1957) reported that 0.5 ppm Mn in Hoagland's nutrient solution was toxic to barley, slightly toxic to lettuce, and non-toxic to tomatoes. Morris and Pierre (1949) found that of five legumes tested, lespedeza and sweet clover were the most sensitive, cowpeas and soybeans were intermediate, and peanuts were the most tolerant to Mn. Manganese concentrations in the leaves ranked in the following order: lespedeza = cowpeas > soybeans > sweetclover = peanuts. Ouelette (1952) stated that levels of water soluble Mn could not exceed 1.0 ppm in the soil for potatoes, 1.5 ppm for clover, 2.0 ppm for lespedeza, and 3.0 ppm for soybeans if good plant growth were to be obtained.

Several theories have been proposed to explain why some plants are more tolerant of Mn than others. Neeman (1960) and Foy _et al._ (1969) suggested it was a plant's ability to tolerate Mn in the tops that allowed it to tolerate high Mn levels. Manganese toxicity symptoms occurred in plant tops at 1000 ppm in beans, 550 ppm in peas, 200 ppm in barley (White, 1970), 3000 ppm in burley tobacco (Ragland and Hiatt, 1963), 5590 ppm in sugar beets (Brown et al., 1968), greater than 600 ppm in coastal bermudagrass (Matocha, 1973), and greater than 8000 ppm in single leaves of rice plants (Tanaka and Navasero, 1966).

Other investigators have related Mn tolerance to reduced Mn transport to the plant tops (Ouelette and Desseseaux, 1958; Andrew and Hegarty, 1969). This could be similar to a tolerance mechanism of some Agrostis species to zinc (Petersen, 1969; Turner and Gregory, 1967; and Turner, 1969 b). Similar amounts of zinc enter the root of tolerant and nontolerant species. Tolerant species bind the excess zinc ions in the root cell walls, rendering it unavailable to the plant tops. Robson and Loneragan (1970) suggested that the sensitivity of Medicago species to Mn was greater than that of Trifolium subterranean because of higher sorption rates and greater transport of Mn from the roots to the tops under luxury conditions. The difference may also be related to their sensitivity to water-logged soils. Doi (1952) studied the oxidizing power of roots under paddy field conditions. He found that Graminae and Compositae had the greatest oxidizing power with less in legumes and vegetables.

A third theory proposed is that some plants "selectively exclude" Mn (Gerloff et al., 1966). It was found that Mn tissue concentrations in different plants growing in an acid bog (pH 4.0) in Wisconsin varied from 71 to 1736 ppm. The conclusion was that a capacity to "selectively exclude" Mn was apparent in environments of low pH. Matocha (1973) found that coastal bermudagrass seemed to accumulate more Mn in plant tissues than Coastcross I bermudagrass under identical soil Mn levels in an acid soil.

MATERIALS AND METHODS

In this study, four experiments were conducted to examine the tolerance of Mn by Penncross bentgrass, (*Agrostis palustris* var. Penncross) and to determine the effect of two soil additives on high Mn levels in golf green soils. The first two experiments were conducted in nutrient solutions varying in Mn concentrations. Their purpose was to determine the toxic concentration of Mn to Penncross bentgrass, both in solution and in the plant itself. Plants in the first experiment were unclipped, while plants in the second experiment were clipped weekly to 1.3 cm.

The third and fourth experiments were conducted with golf green soils high in Mn. Their purpose was to determine the effect of lime and P on the availability of Mn in these soils. One extractant was used in the third experiment, and two in the fourth to measure soil Mn. To evaluate the response of Penncross bentgrass to lime and P applications to the soils, top yield was measured in the third experiment, whereas, in the fourth, Mn concentration in the plant tops was measured.

Nutrient solutions used in these experiments were similar to those described by Hoagland. Manganese and P were omitted from nutrient solutions used in soil studies in order to more accurately evaluate soil Mn-P interactions. In sand cultures, Mn was added as $MnCl_2$ in varying amounts to establish the treatments. Approximately 5.0 ppm Fe was added as $FeNaO_{10}H_{14}O_8N_2H_2O$ (FeNaEDTA) at each nutrient solution application. The pH of nutrient solutions used in soil experiments was 5.2, whereas in sand cultures it was adjusted to 4.5 with dilute HCl.

Plant materials were analyzed for calcium (Ca), magnesium (Mg), iron (Fe), manganese (Mn), and zinc (Zn) using atomic absorption. Samples were prepared using a dry ashing technique, with the dry ash taken up in dilute HCl.

All experiments in this study were arranged in a randomized complete block design. Means were compared using Duncan's New Multiple Range Test at the 5% level of probability. In this study, references to differences between treatments were significantly different at the 5% level of probability, unless otherwise mentioned. The Maximum R^2 Improvement Test was to determine which variables in a collection of independent variables most affected a specific dependent variable.

Experiment I

The purpose of this experiment was to determine the toxic concentration of solution Mn and plant Mn to Penncross bentgrass. Manganese chloride was added to nutrient solution to establish nine levels of Mn: 0.1, 1.0, 2.0, 4.0, 8.0, 16.0, 32.0, 64.0, and 128.0 ppm. Clay pots 16 cm in diameter with a volume of approximately 1400 cc were lined with plastic bags with holes for drainage, and filled with washed quartz sand. Penncross bentgrass was seeded at a rate of 0.975 kg/a (a = are = 100 m^2). Nutrient solution was applied at seeding, and afterwards every third day. It was determined that one pot would hold 500-550 ml of nutrient solution after gravitational water had drained away. To insure there would be no nutrient buildup in the pots, 750 ml of nutrient solution was applied per pot so nutrients remaining from the previous application would be leached out. Treatments were randomized within four replications and placed on a greenhouse bench. Day temperatures were maintained between 75 - 90° F. Night temperature was set at a 60°F minimum. Plants were periodically examined for toxic symptoms. Three months after seeding, plant tops and roots were harvested, weighed, and analyzed for Mn.

Experiment II

In this experiment Penncross was grown under a clipping stress to partially simulate golf green conditions, and to determine what effect, if any, clipping had on the toxic concentration of Mn to the grass. Manganese chloride was added to nutrient solution to establish eight levels of Mn: 4.0, 8.0, 16.0, 32.0, 64.0, 128.0, 256.0, and 512.0 ppm. Sand culture techniques were as described in Experiment I. Penncross bentgrass was seeded at a rate of 0.975 kg/a on August 23. When the plants had grown to a height of 4.0 cm, they were clipped to 1.3 cm, and afterwards clipped to this height once a week. Leaf width was measured using a shadow graph six weeks after seeding. Clippings were weighed and analyzed for Mn eight and ten weeks after seeding. Twelve weeks after seeding, plant tops and roots were harvested and analyzed for Ca, Fe, Mg, Mn, and Zn. The top/root ratio was determined using the accumulated top yield from the three harvests.

Experiment III

The purpose of this experiment was to determine the effect of lime and P on the acid extractable Mn levels in three golf green soils high in Mn, and to evaluate the growth of Penncross bentgrass on the three soils. In this greenhouse study, three soils were selected that were high in Mn. Each was a mixture composed of topsoil from a river terrace or flood plain, sand, and organic matter, combined in ratios suitable for bentgrass golf greens in North Carolina. The topsoil in each mixture came from the following areas: Soil I - river terrace near Thomasville, North Carolina; Soil II - flood plain near Chapel Hill, N. C.; Soil III - river terrace along the Cape Fear River near Moncure, N . C.

Soils I and II were stored in plastic trash cans in the greenhouse approximately six months before use. Soil I was obtained and stored in an air dry state. Soil II was obtained in a completely water saturated state, and remained in this condition throughout the storage period. Soil III, having been obtained several years before, had been fumigated with methyl bromide (CH_3Br) and stored outside under plastic in a dry condition. A soil analysis was performed in duplicate on each soil just prior to the experiment.

On January 25, 1973, clay pots 16 cm in diameter with a volume of approximately 1400 cc were filled with the three soils. Four rates of dolomitic limestone [0, 24.4, 48.8, and 97.6 kg/a (a = are = $100m^2$)] and four rates of P (as P_2O_5) as 20% superphosphate (0, 2.44, 4.88 and 9.76 kg/a) were applied in a factorial design to each soil. Application rates were based on the cross sectional area of the pot, half way from the top to the bottom. Both lime and P were pulverized so that all materials would pass through a 60-mesh screen. They were then mixed with the entire sample of soil in the appropriate pots.

Treatments were randomized within each of three replications and placed on a greenhouse bench. Day temperatures in the greenhouse ranged from 75-90^{o} F, while the night temperature was set at a 60^{o} F minimum. The soils were allowed to incubate for a period of one month, during which time they were watered twice a week to facilitate the lime and P reacting with the soil.

On February 25, the pots were seeded with Penncross bentgrass at a rate of 0.975 kg/a. Plants were fertilized at seedling emergence and afterwards at three week intervals with nutrient solution. Manganese and P were omitted from the nutrient solution used. One month after seeding plant height was measured as the distance from the soil surface to the tip of the tallest leaf. Two months after seeding plant tops were harvested and dry weight measurements taken. Throughout the experiment plant tops were examined for toxic symptoms.

On June 1, soil samples were taken from each pot using a soil sampling tube 1.5 cm in diameter. Samples were taken to a depth of 9.0 cm. Replications were then pooled by treatment and sent to the North Carolina Department of Agriculture, Soil Testing Division, Raleigh, N. C. for analysis. Of importance to this study was the soil Mn extractant used, which was a weak acid composed of approximately 0.05 $\underline{N}$ HCl plus 0.025 $\underline{N}$ H_2SO_4.

Experiment IV

The objective of this experiment was to determine the effect of lime and P on the amount of soil Mn extracted using two extractants, and their effect on the Mn concentration in the tops of Penncross bentgrass. Soil III from Experiment III was chosen for this study. There was an adequate amount of the soil readily available, and it appeared to be the highest in Mn. Clay pots 16 cm in diameter with a volume of approximately 1400 cc were filled with the soil. Three rates of lime as calcium carbonate ($CaCO_3$) (0, 24.4, and 48.8 kg/a) and three rates of P (as P_2O_5) (0, 2.44, and 4.88 kg/a) were applied in a factorial design to the soil. The lime and P were pulverized to pass through a 60-mesh screen, and then mixed with the entire sample of soil in the appropriate pot. Penncross bentgrass was seeded in the pots at a rate of 0.975 kg/a and fertilized with nutrient solution at seedling emergence.

Two weeks after applying the lime and P, soil samples were taken as described in Experiment I, with the exception that replications were not pooled. Soil Mn was extracted using two extractants: 1) weak acid composed of .05 $\underline{N}$ HCl plus .025 $\underline{N}$ H_2SO_4; and 2) 1.0 $\underline{N}$ ammonium acetate (NH_4OAc), pH 7.0. The Mn extracted was measured by atomic absorption. Soil pH was measured using a glass electrode. Three weeks after seeding, plant tops were clipped to 1.3 cm. Two weeks later, plant tops were again clipped to 1.3 cm and the harvested material analyzed for Mn. The plants were then allowed to grow for six weeks before again being clipped and analyzed for Mn.

A comparison of the two soil Mn extractants with the plant Mn concentrations from both harvests was done by linear regression.

RESULTS AND DISCUSSION

Experiment I

In this experiment, Penncross bentgrass was grown under no clipping stress in nutrient solution to determine the toxic level of Mn. Plants grown in nutrient solutions containing 64.0 ppm Mn had a mean concentration of 2343 ppm in the tops. At or above this concentration, toxicity symptoms began to appear on the leaves. White and brown necrotic areas began to develop on the leaf blades. The white areas were not localized on any one part of the leaf. The greatest number of brown spots occurred along the leaf midrib near the tip. Plants exhibiting toxic symptoms also had a noticeable yellowed appearance.

Where plants received 32.0 ppm Mn or more, plant roots turned brown and black, increasing in dark color with increasing Mn levels. The roots appeared to be alive, because they were still intact at harvest. New roots appeared periodically growing down the inside of the pots. In early stages of growth, they were distinguishable from other plant roots by their white color, but later took on the appearance of the other plant roots. The dark color was probably because of high levels of oxidized Mn in the roots. Doi (1952)studied the oxidizing power of plants grown under paddy field conditions. He found the highest oxidizing power in the Graminae family, with less oxidizing power in legumes and vegetable crops.

Manganese had no significant effect on top yield, however, root weights increased with increasing solution Mn levels (Table 1). Since this was a dry weight measurement, the increases were the result of

Table 1. Effect of nutrient solution Mn levels on top dry weight, root dry weight, and top/root ratio of Penncross bentgrass

Mn added	Dry Weight Tops	Dry Weight Roots	Top/Root Ratio
ppm	g/pot		
0.1	43.6a	4.44a	10.29a
1.0	43.7a	4.76ab	9.18ab
2.0	39.0a	4.69ab	8.47abc
4.0	41.8a	5.20abc	8.08abc
8.0	42.9a	5.08abc	9.32ab
16.0	43.1a	5.22abc	8.56abc
32.0	45.5a	6.19bcd	7.50bc
64.0	49.4a	6.67cd	7.43bc
128.0	49.6a	7.34d	6.86c

Means within a column with a common letter are not significantly different at the 5% level according to Duncan's New Multiple Range Test.

more cellular material being present. There are several possible explanations for this increase in root weight. First, the stress caused by excess Mn caused the plants to produce more new roots. Under normal conditions, bentgrass is a profuse root producer. Secondly, the excess Mn caused the roots to swell and consequently be heavier. Visual inspection indicated roots showing toxic symptoms were larger in diameter than roots showing no toxicity. Sartain (1973) found that soybeans grown in acid soils with high aluminum (Al) contents produced heavier root masses and more large roots, but less active root surface than plants grown in soils with a neutral pH and low Al content. He attributed the increase in weight to suberization (conversion of cell walls into corky tissue by infiltration with suberin) occurring in the root tissue. Perhaps this also occurred in the bentgrass roots. Third, the high Mn levels caused an increase in cellular material to increase storage capacity in the roots. Figures 1 and 2 indicated that as solution Mn increased, storage of Mn in the roots also increased.

As expected, both Mn concentration and total Mn increased in plant tops and roots with increasing solution Mn (Table 2). The plants appeared to be able to store some Mn in the tops. Whereas, total top Mn increased, top yield did not. When the resulting increase in top Mn concentration reached 2343 ppm Mn, toxic symptoms began to appear. Turner (1969 a) found that certain *Agrostis* species growing on mine spoils were capable of storing excess Zn and Cu in the plant tops. Increased total root Mn was due to increased root weights and the oxidation and storage of Mn in the root tissues.

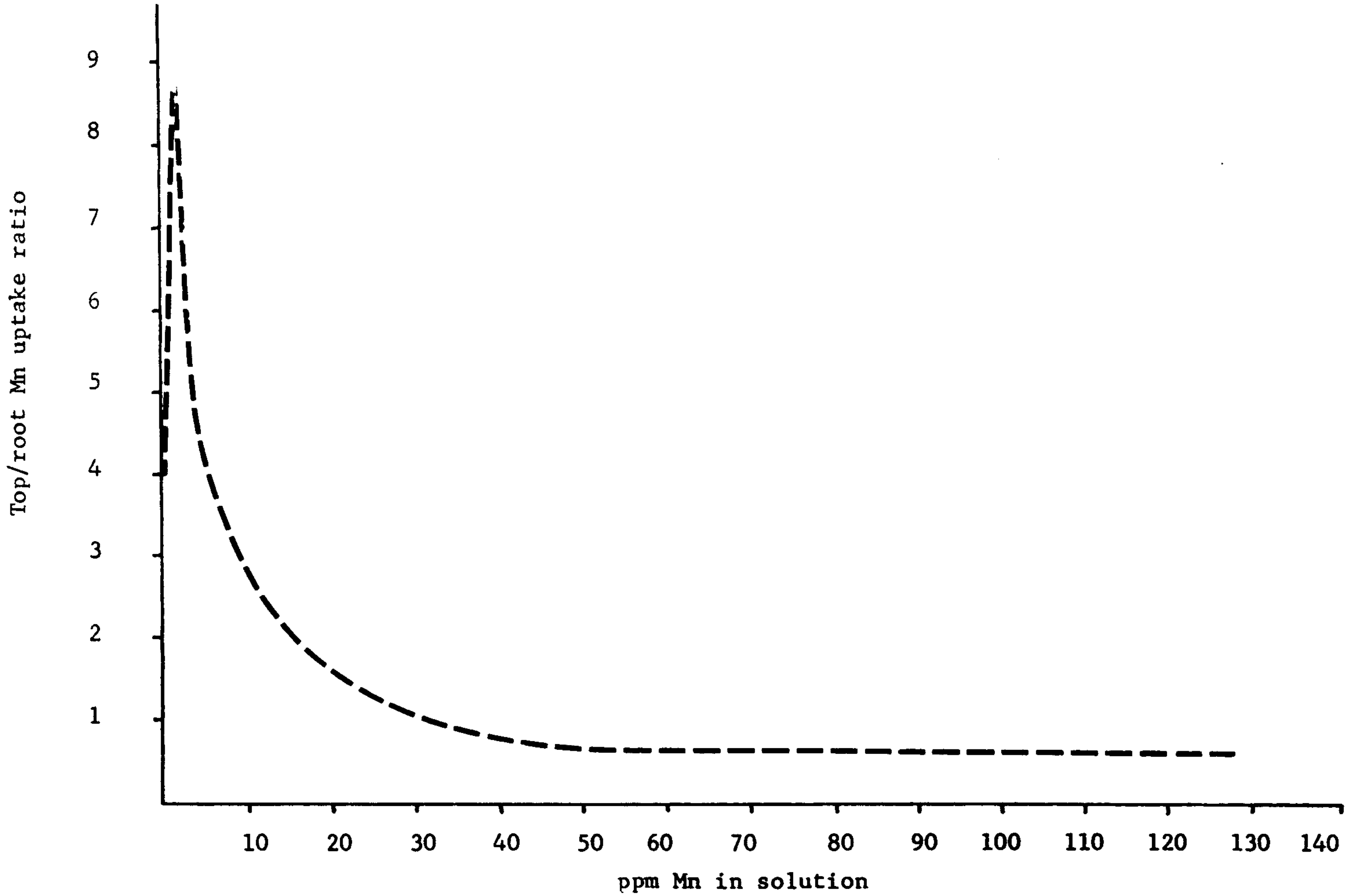

Figure 1. Effect of nutrient solution Mn levels on the top/root Mn uptake ratio of Penncross bentgrass

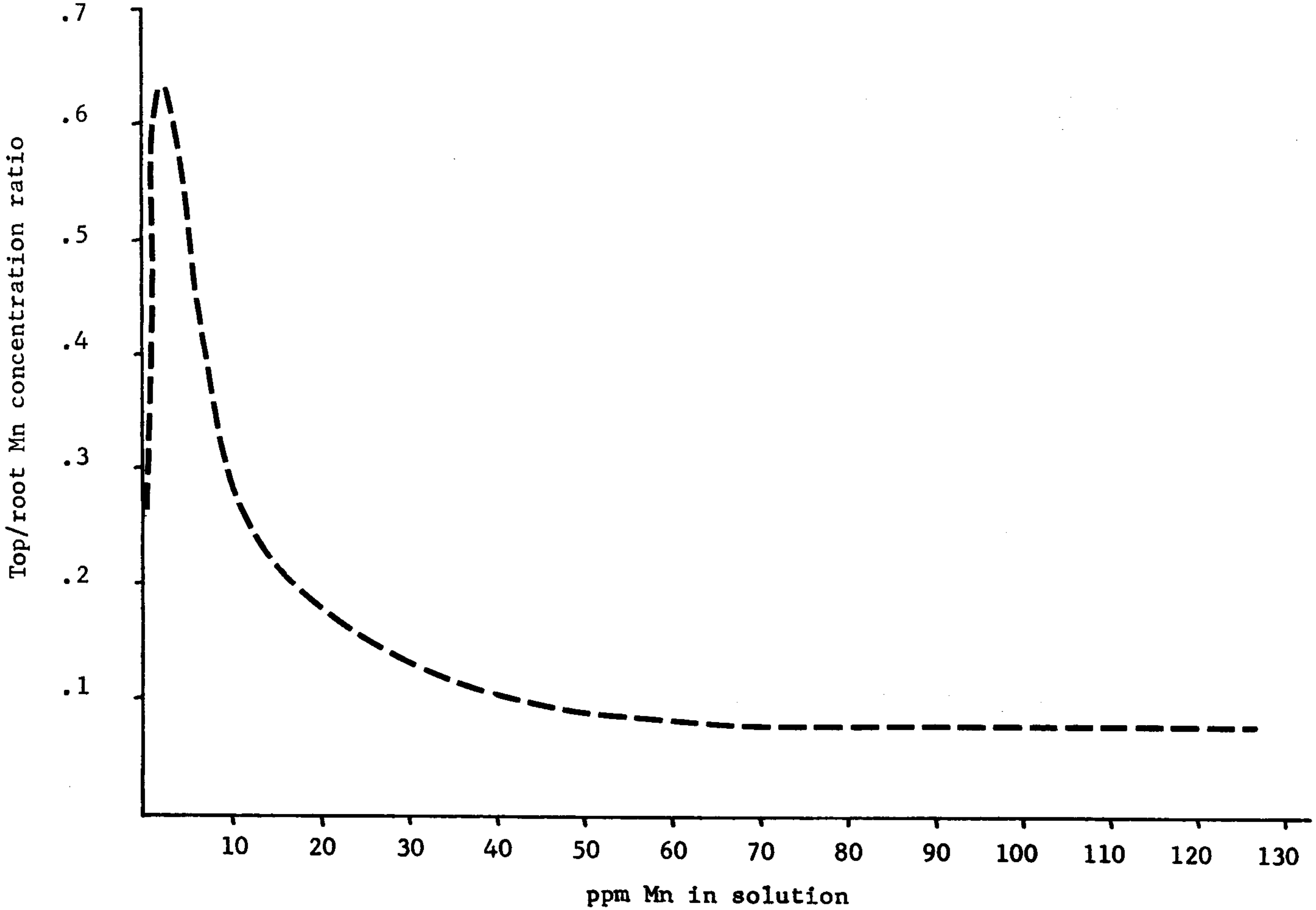

Figure 2. Effect of nutrient solution Mn levels on the top/root Mn concentration ratio of Penncross bentgrass

Table 2. Effect of nutrient solution Mn levels on the Mn concentration and total Mn uptake in the tops and roots of Penncross bentgrass

Mn added	Tops		Roots	
ppm	ppm	ug/pot	ppm	ug/pot
0.1	105a	4625a	413a	1857a
1.0	307ab	13024ab	521a	2589a
2.0	423ab	16212bc	665a	2956a
4.0	549bc	22634bc	898ab	4610a
8.0	845cd	35976d	2478ab	9536a
16.0	1189d	51195e	5834b	29397a
32.0	1902e	83670f	14544c	84798b
64.0	2343f	114219g	28734d	187944c
128.0	3076g	150078h	36208e	268731d

Means within a column with a common letter are not significantly different at the 5% level according to Duncan's New Multiple Range Test.

Under luxury conditions Mn uptake does not appear to be as much a function of plant growth as does Mn concentration in solution.

The top/root ratio decreased with increasing solution Mn (Table 1). The lowest Mn treatment was significantly higher than the three highest Mn treatments. It is generally believed that Mn affects plant tops more than roots. In this case, where neither top nor root weights decreased, but the top/root ratio did, it is difficult to conclude which was affected most. Toxicity symptoms did appear on the roots at a lower Mn solution level.

The top/root Mn ratio (Figure 1) and the top/root Mn concentration ratio (Figure 2) decreased at 1.0 and 2.0 ppm solution Mn respectively. These ratios indicate that less Mn was being transported to the plant tops per unit of Mn taken up by the roots when solution Mn exceeded 2.0 ppm. Above 2.0 ppm Mn in solution, most excess absorbed Mn was stored in the roots. One ppm Mn was sufficient for good plant growth.

Manganese tolerance in Penncross bentgrass appeared to be related to a high internal tolerance to high Mn levels in the roots, and to a lesser extent in the tops. Beside being able to store Zn and Cu in the tops (Turner 1969 a), Turner and Gregory (1967), and Turner (1969 b) found that some *Agrostis* species stored excess Zn in the root cell walls and thus rendered it unavailable for plant use. Perhaps this is a tolerance mechanism of Penncross bentgrass to Mn.

The tolerance mechanism of Penncross bentgrass to Mn may also be related to its tolerance to high soil moisture conditions.

Foy et al. (1973) concluded from evidence in the literature that "the tolerance of certain species to wet soils coincide with their tolerance to excess Mn in nutrient solutions." Doi (1952) suggested that the tolerance of plant species to wet soils was associated with the oxidizing power of their roots. Grasses were the most effective oxidizers, and therefore might be expected to tolerate more Mn. The ability of bentgrass to oxidize excess Mn in the roots and thus reduce Mn transport to the tops allowed the plants to tolerate high Mn levels in nutrient solution. When the Mn concentration in the tops reached 2343 ppm, toxic symptoms began to appear on the leaves. By this time, however, root discoloration was evident in plants receiving less Mn. Any detrimental effect the excess Mn had on plant growth was evident only in the top/root ratio, which decreased because of the heavier root systems.

Experiment II

The effect of various levels on the growth of Penncross bentgrass clipped weekly to 1.3 cm was examined in this experiment. Severe toxicity symptoms similar to those described in Experiment I were evident on the leaves of clipped plants receiving 32.0 ppm Mn in solution. In unclipped plants, it took twice that amount for toxic symptoms to appear. The tops of plants receiving 16.0 ppm Mn or greater were yellowed in appearance. Three to five days after clipping, portions of the remaining plant tops that had shown toxicity appeared brown and dead. Regrowth from these areas was not evident. Root discoloration was evident at 8.0 ppm Mn in solution.

At high Mn levels, plant tops appeared slow in developing. Measurement of leaf widths indicated that as solution Mn increased, the width of plant leaves decreased (Table 3).

As Mn concentrations in solution increased, top yield at all harvest dates decreased (Table 4). As top yields decreased, top Mn concentrations (Table 5) and total top Mn (Table 6) increased. This suggests that a portion of the Mn translocated to the plant tops was being stored in these tissues. Turner (1969 a) reported that Colonial bentgrass was able to immobilize Cu and Zn in the cell walls of leaves. This way, toxic levels of these elements did not build up at metabolic sites.

Top Mn concentrations were greater in the regularly clipped plants (Experiment II) than in the unclipped plants (Experiment I) at equal Mn solution levels. Age and type of tissue could have caused the difference. Clipped plant material was younger and contained a higher percentage of leaf blade tissue and a lower percentage of stem tissue than the unclipped plants. Accumulation of Mn may have been greater in the leaf blades than in the stems.

Root weights of plants grown in solution containing 64.0, 128.0, and 256.0 ppm Mn were higher than all others (Table 7). The increase was probably caused by roots swelling and increased cellular material, as explained in Experiment I. Root Mn concentration and total root Mn increased with increasing solution Mn (Table 7). Root Mn concentrations were two to three times higher in the clipped plants than in the unclipped plants at equal Mn solution levels. Root discoloration appeared at approximately the same root Mn concentrations in clipped and unclipped plants.

Table 3. Effect of nutrient solution Mn levels on the leaf width of Penncross bentgrass

Mn added	Leaf width
ppm	mm
4.0	1.71a
8.0	1.67ab
16.0	1.70ab
32.0	1.57ab
64.0	1.43ab
128.0	1.22c
256.0	1.20c
512.0	1.15c

Means within a column with a common letter are not significantly different at the 5% level according to Duncan's New Multipe Range Test.

Table 4. Effect of nutrient solution Mn levels on the top dry weight of Penncross bentgrass at three harvest dates

Mn added	Harvest Dates Oct. 9	Oct. 23	Nov. 6	Total
ppm	Top dry weight (g/pot)			
4.0	3.03ab	3.24a	2.20a	8.47a
8.0	2.90ab	2.92ab	1.86ab	7.69ab
16.0	3.37a	2.77ab	1.88ab	8.03ab
32.0	2.80ab	2.51bc	1.72ab	7.03bc
64.0	2.51ab	2.44bcd	1.35b	6.31cd
128.0	2.27abc	2.07cde	1.34b	5.62d
256.0	2.13bc	1.94de	1.28b	5.32de
512.0	1.31c	1.73e	1.26b	4.36e

Means within a column with a common letter are not significantly different at the 5% level according to Duncan's New Multiple Range Test.

Table 5. Effect of nutrient solution Mn levels on the concentration of Mn in the dry plant tops of Penncross bentgrass at three harvest dates

Mn added	Harvest Dates		
	Oct. 9	Oct. 23	Nov. 6
ppm	Top Mn (ppm)		
4.0	1473a	762a	766a
8.0	1751a	1275a	930a
16.0	2160a	1459a	1379a
32.0	2993a	2461b	2729b
64.0	4546b	4126c	3448bc
128.0	6210c	4388cd	3968cd
256.0	8636d	5255d	4661de
512.0	11179e	5120d	5482e

Means within a column with a common letter are not significantly different at the 5% level according to Duncan's New Multiple Range Test.

Table 6. Effect of nutrient solution Mn levels on the total Mn in the dry plant tops of Penncross bentgrass at three harvest dates

Mn added	Harvest Dates Oct. 9	Oct. 23	Nov. 6	Total
ppm	———Mn uptake (ug/pot)———			
4.0	4544a	2461a	1694a	8700a
8.0	5076a	3731a	1797a	10604a
16.0	7160ab	4090a	2601a	13852ab
32.0	8318abc	6014b	4336b	18669bc
64.0	11346abcd	10059c	4670b	26076cd
128.0	13612bcd	9078c	5059bc	27750d
256.0	18309d	10169c	5853c	34333d
512.0	15502cd	8785c	7135d	31423d

Means within a column with a common letter are not significantly different at the 5% level according to Duncan's New Multiple Range Test.

Table 7. Effect of nutrient solution Mn levels on root weight, root Mn concentration, and total root Mn in Penncross bentgrass

Mn added	Root weight grams	Root Mn conc.	Total Root Mn
ppm	g/pot	ppm	ug/pot
4.0	1.27a	5596a	7331a
8.0	1.44a	11695a	16740a
16.0	1.65a	14471a	24730ab
32.0	1.70a	35306b	59436b
64.0	2.30b	59698c	162143c
128.0	2.19b	75144cd	135345c
256.0	2.36b	58409c	135033c
512.0	1.41a	90236d	127611c

Means within a column with a common letter are not significantly different at the 5% level according to Duncan's New Multiple Range Test.

The top/root ratio (Figure 3) and the top/root Mn ratio (Figure 4) decreased with increasing solution Mn levels. In both cases, the lowest Mn treatment was significantly higher than all others. It appeared that high Mn levels were more detrimental to top growth than root growth when the plants were under a clipping stress.

Plant materials from the last harvest were analyzed for Fe, Ca, Mg, and Zn. Concentrations, totals, and the top/root ratios of these elements were measured. Both the top Fe concentration (Table 8) and the total top Fe (Table 9) decreased sharply when solution Mn exceeded 16.0 ppm Mn. Shive (1941) and Sommers and Shive (1944) reported that high levels of tissue Mn were associated with low Fe tissue concentrations. Snyder and Gascho (1973) found that increasing available Fe reduced Mn toxicity symptoms in St. Augustine grass. There were no significant changes in root Fe concentrations (Table 10), total root Fe (Table 11), or the top/root Fe ratio (Table 12). There was a general increase in total root Fe with increasing solution Mn. The reduction in top Fe concentration plus no significant change in root Fe concentration suggest a change in Fe transport from the roots to the tops. Reikels and Lingle (1966) and Epstein and Stout (1951) reported that high levels of Mn in solution inhibited uptake and transport of Fe in tomatoes. Perhaps the large amounts of Mn in the bentgrass roots complexed or immobilized the Fe in the roots, or else competed with it for transport to the plant tops. The swelling of the roots could have been a major factor in this.

There were reductions in Ca and Mg concentrations (Table 8) and totals (Table 9) in the plant tops with increasing solution Mn. The

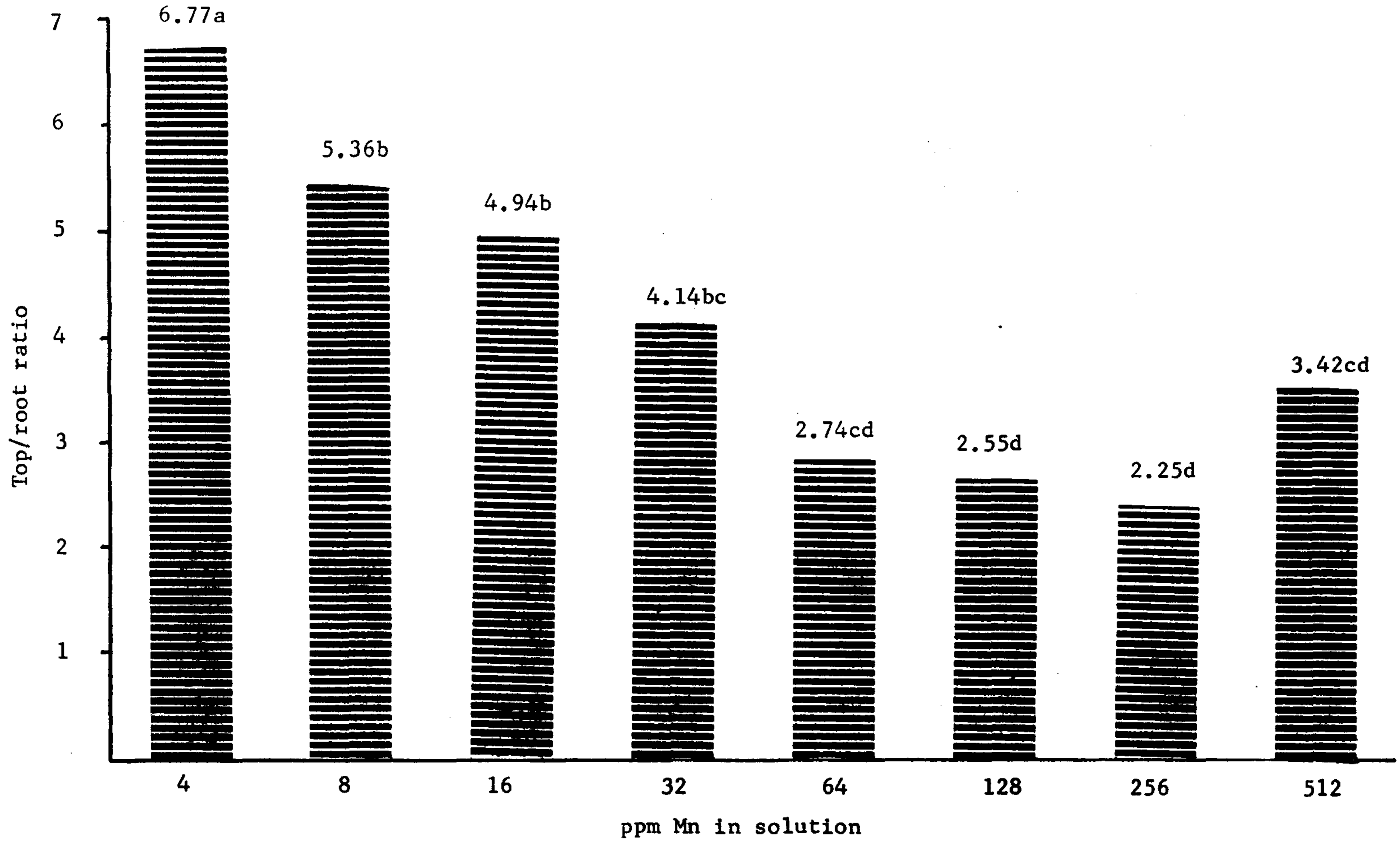

Figure 3. Effect of nutrient solution Mn levels on the top/root ratio of Penncross bentgrass clipped weekly to 1.3 cm.

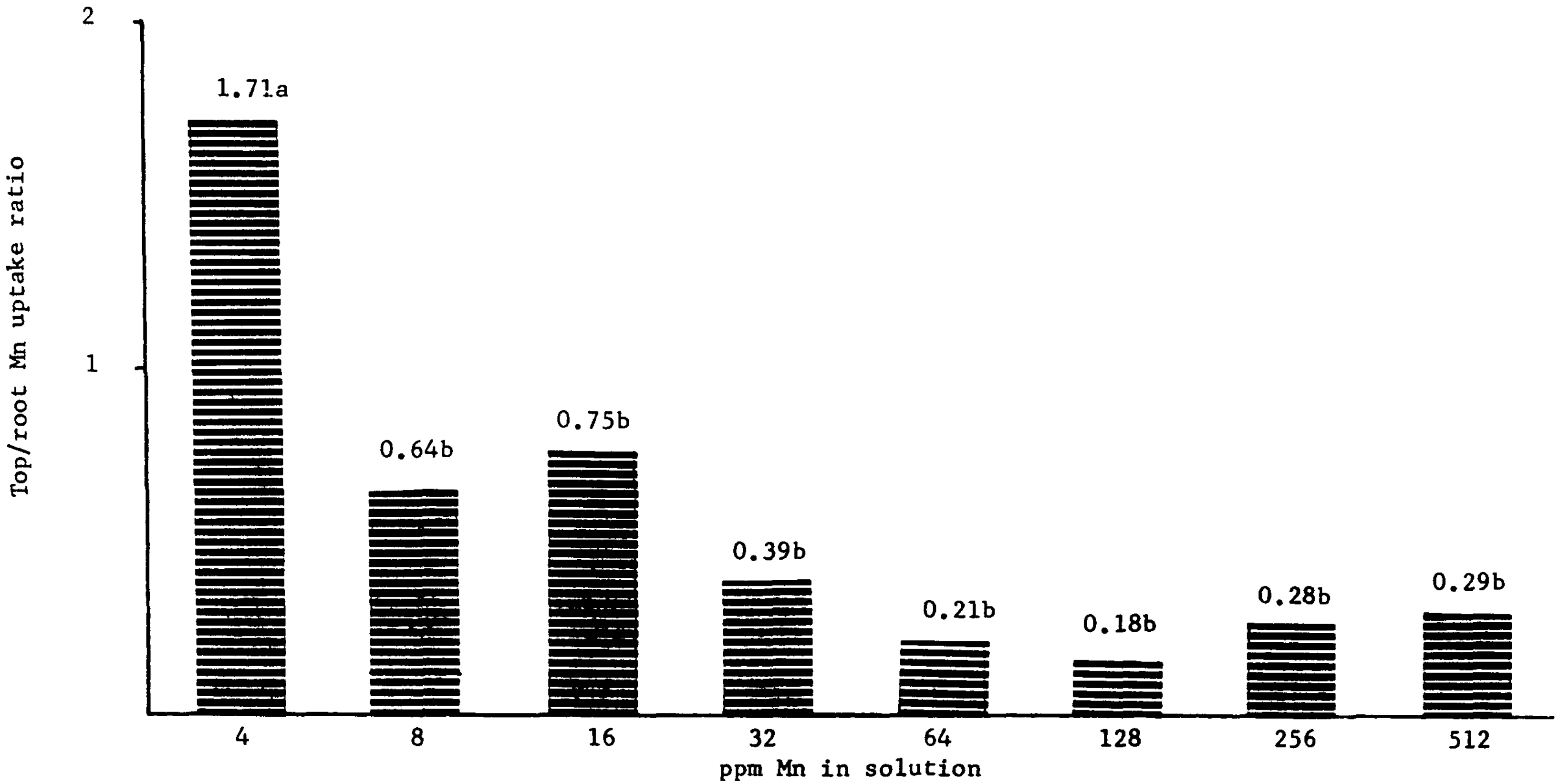

Figure 4. Effect of nutrient solution Mn levels on the top/root Mn uptake ratio of Penncross bentgrass clipped weekly to 1.3 cm.

Table 8. Effect of nutrient solution Mn levels on the concentrations of Fe, Ca, Mg, and Zn in the dry plant tops of Penncross Bentgrass

Mn added	Fe	Ca	Mg	Zn
ppm	ppm			
4.0	83a	5404a	4419a	47ab
8.0	81a	5519a	4255a	65bcd
16.0	81a	5283a	4436a	34a
32.0	43ab	5453a	4586a	53abc
64.0	48ab	5061ab	3161b	80cd
128.0	16b	4417b	2920bc	78cd
256.0	26b	4379b	2950bc	112e
512.0	41ab	3532c	2624c	89de

Means within a column with a common letter are not significantly different at the 5% level according to Duncan's New Multiple Range Test.

Table 9. Effect of nutrient solution Mn levels on the total Fe, Ca, Mg, and Zn in the dry plant tops of Penncross bentgrass

Mn added	Fe	Ca	Mg	Zn
ppm	ug/pot			
4.0	184a	11876a	9700a	108ab
8.0	146abc	10396a	8025a	114ab
16.0	153ab	9954ab	8353a	64b
32.0	86bcd	9072ab	7706a	89ab
64.0	64cd	6851bc	4279b	109ab
128.0	22d	5614c	3725b	100ab
256.0	34d	5507c	3712b	139a
512.0	52d	4644c	3455b	117ab

Means within a column with a common letter are not significantly different at the 5% level according to Duncan's New Multiple Range Test.

Table 10. Effect of nutrient solution Mn levels on the concentration of Fe, Ca, Mg, and Zn in the dry plant roots of Penncross bentgrass

Mn added	Fe	Ca	Mg	Zn
ppm	ppm			
4.0	1340a	7713a	2028bc	145a
8.0	1771a	10905ab	2166b	216a
16.0	1849a	14885bc	2816a	142a
32.0	1838a	15485c	2139b	215a
64.0	1858a	14535bc	1600bcd	201a
128.0	2016a	10096a	1359cd	159a
256.0	1615a	7160a	1080d	226a
512.0	3396a	8253a	1566bcd	368a

Means within a column with a common letter are not significantly different at the 5% level according to Duncan's New Multiple Range Test.

Table 11. Effect of nutrient solution Mn levels on the total Fe, Ca, Mg, and Zn in the dry roots of Penncross bentgrass

Mn added	Fe	Ca	Mg	Zn
ppm		ug/pot		
4.0	1812a	9778d	2553bc	196a
8.0	2593a	15678cd	3122bc	313a
16.0	3120a	24646abc	4639a	228a
32.0	3150a	26346ab	3620ab	368a
64.0	4354a	33481a	3641ab	476a
128.0	4195a	22195bc	2973bc	357a
256.0	3614a	16888cd	2507bc	543a
512.0	4006a	11703d	2231c	457a

Means within a column with a common letter are not significantly different at the 5% level according to Duncan's New Multiple Range Test.

Table 12. Effect of nutrient solution Mn levels on the top/root ratios of Fe, Ca, Mg, and Zn in Penncross bentgrass

Mn added	Fe	Ca	Mg	Zn
ppm	Top/Root Ratio			
4.0	.15a	1.21a	3.79a	.95a
8.0	.06a	.78bc	2.87ab	.67a
16.0	.09a	.78bc	1.86bc	.29a
32.0	.05a	.36bc	2.12bc	.30a
64.0	.14a	.21c	1.20c	.29a
128.0	.06a	.26c	1.26c	.30a
256.0	.06a	.34c	1.51bc	.30a
512.0	.01a	.43bc	1.69bc	.27a

Means within a column with a common letter are not significantly different at the 5% level according to Duncan's New Multiple Range Test.

root Ca concentration (Table 10) and total root Ca (Table 11) increased up to 32.0 and 64.0 ppm Mn in solution respectively, and then decreased. Root Mg concentrations (Table 10) and total root Mg (Table 11) increased to 16.0 ppm of solution Mn and then decreased. The top/root ratios of Ca and Mg (Table 12) were highest at the lowest Mn treatment.

The reductions in top Ca and Mg concentrations and the top/root ratios indicated a decrease in transport of these ions to the plant tops. There are several possible explanations for this. The Ca and Mg ions could have been complexed in the roots in some manner so their transport to the tops was inhibited. The Mn could have caused a breakdown in cellular structure in the roots which led to a blockage of transport pathways. Increasing Mn concentrations resulted in brown and black roots, which may have been either oxidized Mn or dead tissues or both. Mutual competition for transport could have existed between the Ca, Mg, and Mn. The three ions are similar in molecular structure and may be transported by the same mechanism in the plant. This mechanism could have become increasingly saturated with Mn, thus reducing Ca and Mg transport. Since most ion transport is controlled by metabolic processes, the decreased top growth could have caused the reductions in Ca and Mg.

Reductions in root Ca and Mg occurred almost simultaneously with increases in root weights. The high Mn concentrations, which probably caused the increase in root weight, apparently also affected Ca and Mg uptake. Mass et al. (1969) said that Mn had no effect on Ca uptake in excised barley roots. It should be noted, however, that a negligible amount of Ca was absorbed by the barley roots at any Mn level. Manganese had a depressive effect on Mg uptake by the barley roots. At equivalent concentrations (5.0 meq./L.), Mg uptake was reduced to only 8% of its rate when Mn was excluded from the solution. At equivalent concentrations, Mn uptake was four times greater than Mg uptake. Mass et al. (1969) said there was no evidence for mutual competition between the ions for uptake, and suggested that changes in carrier molecules were responsible for the changes in the ion levels. They concluded that many of the interacting effects between the cations occurred at points other than at the actual transport site.

Manganese appeared to have little effect on Zn uptake. Zinc concentrations in the plant tops (Table 8) were higher at high Mn levels. Root Zn concentrations (Table 10) and total top and root Zn levels (Tables 9 and 11) showed little or no significant changes.

A Maximum R^2 Improvement test was used to determine which variables in a collection of independent variables should be used in a regression model for the dependent variables total top weight, root weight, and the top/root ratio. The independent variables were separated into two different groups: 1) the concentrations of Mn, Ca, Fe, Mg, and Zn; 2) total amounts of Mn, Ca, Fe, Mg, and Zn. Each group included top and root measurements. Each dependent variable was then regressed against both groups of independent variables, and the best regression model for each dependent variable chosen (Table 13). The first variable in each model was rated the most important for that dependent variable. It explained the highest percentage of the

Table 13. Regression models for top yield, root yield, and top/root ratios

Dependent Variable	Regression Model	R^2
Total Top Yield	$4.2272 + .000462^{**}X_1 - .000621^{**}X_2 + .000099^{**}X_3$	.82
Root Yield	$2.436 - .000802^{**}X_4 = .0000949^{**}X_5 - .000114^{**}X_6$	.71
Top/Root Ratio	$4.8859 - .000331^{**}X_7 - .000344^{**}X_5 + .001889^{**}X_4 + .00519^{**}X_8$	.78

** - Highly significant

X_1 = Total top Mg

X_2 = Total top Mn, third harvest

X_3 = Total Mn uptake into tops

X_4 = Concentration root Mg

X_5 = Concentration root Ca

X_6 = Concentration root Fe

X_7 = Concentration top Mn, first harvest

X_8 = Concentration root Zn

changes in that particular dependent variable. The models indicated that a number of independent variables other than Mn had a significant effect on the dependent variables. It appeared that Mn had a greater effect on top growth than either the root growth or the top/root ratios. Since elements other than Mn were measured only once, these models should be viewed cautiously. For example, there were no significant changes in root Zn concentrations. Yet when it was combined with all other element concentrations, the model indicated that it had a significant effect in the top/root ratio.

Experiment III

In this experiment, the effect of applications of lime and P on acid-extractable Mn levels in three golf green soils was examined. The acid extractant used was composed of approximately 0.05 N HCl plus 0.025 N H_2SO_4. Manganese removed from the soil using this extractant would include Mn^{+2} in solution and on the clay surface, and the Mn^{+3} and Mn^{+4} compounds easily reduced to Mn^{+2}. Tables 14, 15, and 16 show general chemical characteristics of the three soils after applications of lime and P. An overall treatment mean of the three soils showed that liming generally increased acid-extractable Mn levels, whereas P had little effect (Figure 5). Considerable evidence exists showing that liming will reduce Mn availability in soils, however, this effect is mainly on the smaller fraction of readily available or water soluble Mn in the soil, whose availability is controlled by pH. Availability of the larger amount of acid extractable Mn is probably controlled more by total soil Mn. Any effect higher pH values had on acid-extractable Mn were masked by

Table 14. Effect of applications of lime and phosphorous on some chemical properties of soil I

Treatments							
Lime	P	pH	P	K	Ca	Mg	Mn
Kg/a 1|			Kg/a				
Check		6.0	1.20	.72	12.4	1.99	.60
00.0	2.44	6.1	1.80	.69	12.6	1.77	.73
00.0	4.88	6.1	2.10	.69	13.6	1.65	.96
00.0	9.76	6.2	3.15	.53	14.4	1.46	.37
24.4	0.00	6.6	1.47	.56	18.0	5.83	.74
24.4	2.44	6.8	2.73	.59	19.4	6.12	.82
24.4	4.88	6.5	3.36	.59	26.4	5.83	.74
24.4	9.76	6.6	4.20	.63	26.4	5.39	.83
48.8	0.00	6.9	1.38	.53	19.2	6.71	.80
48.8	2.44	7.0	2.94	.59	26.4	7.29	.98
48.8	4.88	7.0	3.36	.53	27.6	7.44	.83
48.8	9.76	6.9	4.62	.69	33.6	7.44	.88
97.6	0.00	6.9	1.44	.59	27.6	9.04	1.00
97.6	2.44	7.0	1.95	.50	26.4	7.87	.88
97.6	4.88	7.1	2.55	.59	32.4	8.89	.90
97.6	9.76	7.0	3.30	.59	27.6	7.44	.92
Check*		5.7	1.21	.84	13.8	2.79	2.47

* - Soil analysis values from check pots taken before experiment

1| a = are = 100 m^2

Table 15. Effect of applications of lime and phosphorous on some chemical properties of soil II

Treatments							
Lime	P	pH	P	K	Ca	Mg	Mn
Kg/a			Kg/a				
Check		6.6	.30	.69	14.4	5.54	.80
00.0	2.44	6.5	.87	.66	15.4	4.67	.69
00.0	4.88	6.7	2.25	.78	17.6	4.52	.83
00.0	9.76	6.6	3.78	.84	18.6	2.38	.78
24.4	0.00	6.9	.36	.75	16.0	6.42	.51
24.4	2.44	6.5	1.05	.88	17.6	5.98	.90
24.4	4.88	6.8	2.94	.94	19.6	5.83	.99
24.4	9.76	6.8	4.41	.94	25.2	4.96	.96
48.4	0.00	7.1	.42	.75	18.2	7.29	1.06
48.8	2.44	7.2	1.11	.84	19.8	6.71	1.07
48.8	4.88	7.1	2.94	.88	25.2	6.56	.88
48.8	9.76	7.0	4.62	.97	27.6	6.27	1.12
97.6	0.00	7.1	.27	.78	25.2	8.60	1.04
97.6	2.44	7.1	.96	.75	25.2	8.02	1.10
97.6	4.88	7.2	1.80	.75	26.4	7.58	1.04
97.6	9.76	7.2	3.45	1.09	25.2	6.57	1.04
Check*		6.3	.12	1.00	14.8	5.10	2.43

* - Soil analysis values from check pots taken before experiment

Table 16. Effect of applications of lime and phosphorous on some chemical properties of soil III

Treatments							
Lime	P	pH	P	K	Ca	Mg	Mn
Kg/a			Kg/a				
Check		6.2	.90	1.03	4.40	.83	.78
00.0	2.44	6.0	1.48	1.09	6.40	.70	.75
00.0	4.88	6.3	2.55	1.47	9.60	.83	.72
00.0	9.76	6.3	3.76	1.47	7.60	.70	.75
24.4	0.00	6.9	1.47	1.13	13.40	5.10	.58
24.4	2.44	6.5	2.94	1.60	13.60	4.67	.94
24.4	4.88	6.8	3.99	1.19	15.40	4.96	1.01
24.4	9.76	6.8	5.05	1.66	15.60	4.37	1.04
48.8	0.00	7.1	1.47	1.53	14.00	5.54	1.01
48.8	2.44	7.1	3.15	1.31	15.20	5.54	.85
48.8	4.88	7.1	3.99	1.63	17.60	5.83	1.04
48.8	9.76	6.7	4.62	1.06	18.80	5.69	.98
97.6	0.00	7.0	1.20	1.13	16.00	6.56	1.15
97.6	2.44	7.1	2.55	1.16	18.40	7.00	1.12
97.6	4.88	7.1	3.00	1.50	19.80	7.00	1.15
97.6	9.76	7.1	4.20	2.35	24.00	6.71	1.02
Check*		5.5	.78	.94	12.00	.80	2.15

* - Soil analysis values from check pots taken before experiment

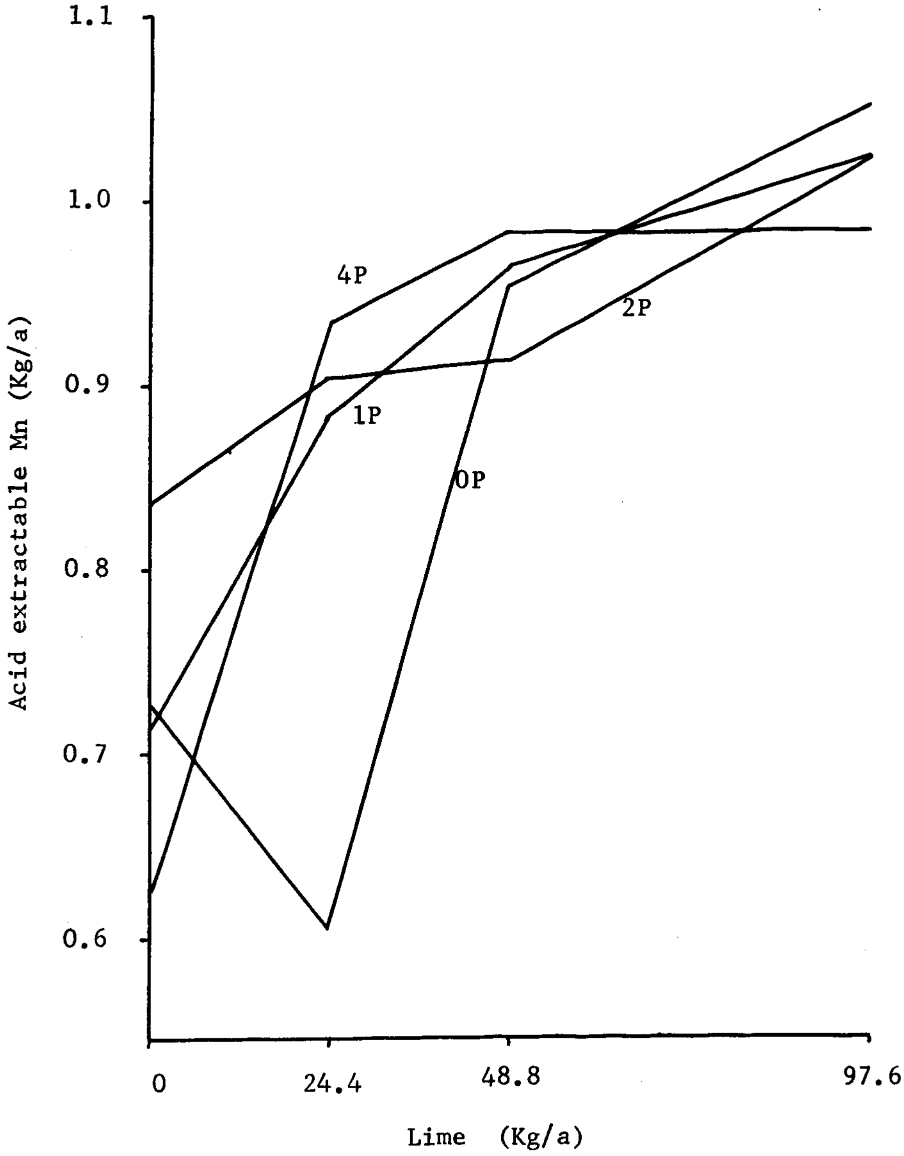

Figure 5. The effect of lime and P on the mean acid-extractable Mn level of three golf green soils.

1P = 2.44 Kg/a P; 2P = 4.88 Kg/a P; 4P = 9.76 Kg/a P the pH of the extractant used. Why Mn levels increased is not clear. Messing (1965) found in well drained soils used in nurseries that applications of lime and P increased the easily-reducible Mn (that Mn extracted with 0.2% hydroquinone in neutral normal NH_4OAc), but decreased the amount of water soluble and NH_4OAc extractable Mn. His reference to easily-reducible Mn would be similar to the acid-extractable Mn in this experiment. Jones (1957 b) reported that liming a Mn deficient Penola soil from pH 7.0 to 8.0 increased Mn uptake in oats.

Acid-extractable Mn levels in the check pots after the experiment were found to have decreased to one-third to one-fourth their value before the experiment (Tables 14, 15, 16). Storage conditions can explain the changes in acid-extractable Mn levels in soils I and II. Soil I was stored in an air-dried condition in the greenhouse for six months before the first soil test. Soil II was stored for a similar length of time in the greenhouse in a water saturated state. Both storage conditions favor the reduction of manganic compounds to Mn^{+2}. Fujimoto and Sherman (1945) found in high Mn soils up to a six fold increase in the exchangeable Mn level after fourteen weeks in an air dry state. They and Boken (1952) reported increases in exchangeable Mn levels in air-dried soils were a function of temperature and length of time in storage. Jones (1957 b) said that in air-dried soils Mn oxidation was completely inhibited, and that the only reaction taking place was the reduction of manganic oxides to Mn^{+2} by organic matter. Graven _et al._ (1965), Meek _et al._(1968), and Bradfield _et al._ (1934) reported that flooded and anaerobic conditions in soils increased exchangeable soil Mn and plant Mn concentrations. When soil samples from the check pots were first taken, both soils were high in exchangeable Mn. Subsequent exposure of the soils to oxidizing conditions in the pots decreased the exchangeable Mn to more normal levels.

Soil III was originally high in acid-extractable Mn because of fumigation with methyl bromide several years before. Timonin (1946) and Jones (1957 a) found that fumigating and sterilizing soils resulted in an increase in availability of soil Mn. Smith (1963) reported significant increases in NH_4OAc extractable Mn thirty months after fumigation of fields with methyl bromide. Fumigation increases soil Mn availability by killing oxidizing microorganisms in the soil and creating a reducing condition. Storage of soil III under plastic after fumigation probably extended the length of time the methyl bromide had an effect on the Mn availability. Exchangeable Mn levels decreased when the soil was exposed to air, water, and microorganisms in the pot.

No toxicity symptoms were seen on the leaves of plants grown in the three soils. It should be noted, however, the plants were grown under greenhouse conditions with no clipping stress. If grown under golf green conditions, where a reducing environment favors increased Mn availability and the grass is clipped almost daily, toxicity would have been more likely. A recent soil test from golf greens constructed eight years ago from soil III indicated that acid-extractable Mn levels in the top 7.2 cm were approximately 1.2 kg/a. These samples were taken from areas in the greens where suspected Mn toxicity symptoms were evident on the grass. The Mn levels in these areas compared favorably with the Mn values found in Table 16.

There were no significant differences between treatments in top yield on any of the individual soils, however, the top yield treatment means for the three soils tended to increase with applications of lime (Figure 6). Responses to applications of P were negligible. Schnehl *et al.* (1950) found that Mn toxicity did not reduce alfalfa yields until toxicity symptoms became severe.

Experiment IV

In this experiment, Mn extracted with 1.0 N NH_4OAc, pH 7.0 was called exchangeable Mn. This would be predominately Mn^{+2} ions in solution and on the clay surface. Manganese extracted with the weak acid was called acid-extractable, as in Experiment III. There were no significant differences between treatments in acid extractable Mn levels (Table 17). There was a trend of P increasing acid-extractable Mn levels at 0 and 24.4. kg/a of lime and decreasing it at 48.8 kg/a of lime. Highest acid extractable Mn levels were found at the highest rates of lime, which is similar to the response found in Experiment III. The acid extractant made the pH lower during extraction procedures, thus there was little or no liming effect. Exchangeable Mn levels were over four times greater when no lime was applied than when 24.4 kg/a of lime was applied, regardless of the P level (Figure 7). Although not significant, at the highest rate of lime, exchangeable Mn levels were over five times greater when P was omitted than when P was applied at the high or low rate.

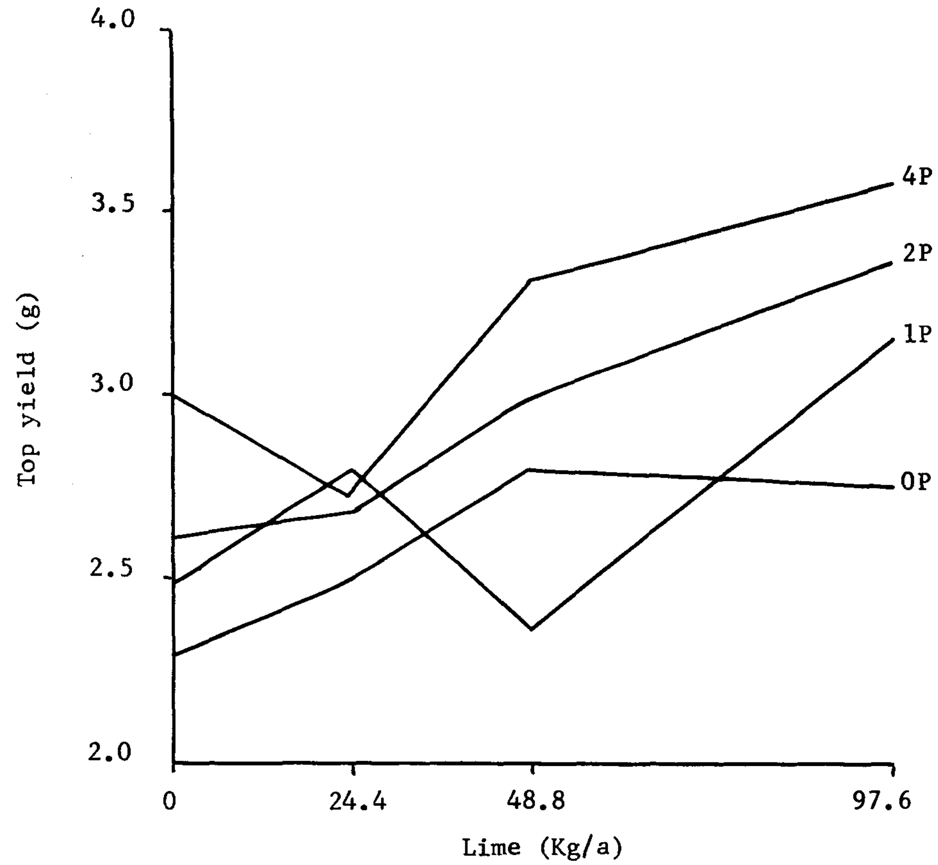

Figure 6. The effect of lime and P on the mean top yield of Penncross bentgrass grown on three soils.

1P = 2.44 Kg/a; 2P = 4.88 Kg/a; 4P = 9.76 Kg/a

Table 17. The effect of applications of lime and P on the amount of exchangeable and extractable Mn in soil III.

Treatments			Soil Mn	
			Extractable	Exchangeable
Lime	P	Soil pH	(weak acid)	(NH_4OAc)
Kg/a			Mn (ppm)	
00.0	0.00	5.6	50.0a	5.36abc
00.0	2.44	5.6	54.5a	8.20a
00.0	4.88	5.6	54.6a	9.47a
24.4	0.00	6.3	46.1a	1.33bc
24.4	2.44	6.3	49.0a	1.33bc
24.4	4.88	6.4	53.2a	1.40bc
48.8	0.00	6.8	59.4a	5.50abc
48.8	2.44	6.8	56.3a	0.97bc
48.8	4.88	6.8	53.9a	0.70c
00.0*	0.00*	5.3	133.3	81.4

* - Analysis from sample not put in pots, sample taken at same time as samples from pots.

Means within a column with a common letter are not significantly different at the 5% level according to Duncan's New Multipe Range Test.

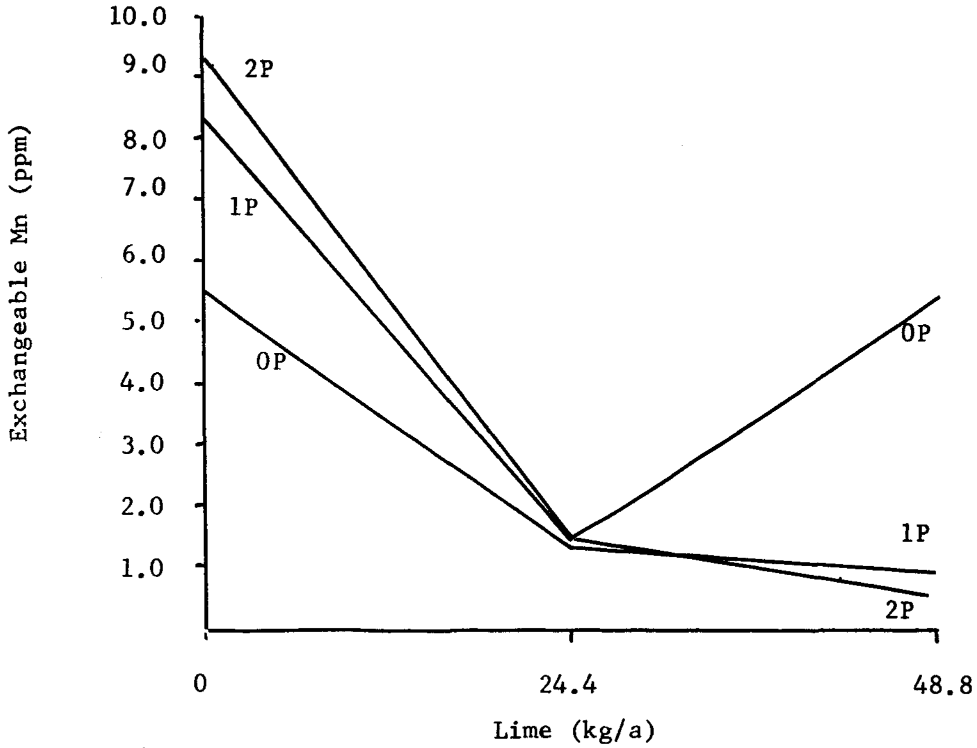

Figure 7. Effect of lime and P on the amout of exchangeable Mn in soil III.
1P = 2.44 kg/a P; 2P = 4.88 kg/a P

These results were somewhat in conflict with those found by Page et al. (1962). They found that applications of P in acid soils reduced available Mn levels and in neutral soils increased them. The difference could be in the soil pH. They used a neutral soil and one with a pH less than 5.0. In this experiment the soil had a pH greater than 5.5. The amounts of Mn in each of its three valence states could also affect the results. Page et al. (1962) offered no conclusions as to which form of Mn was most affected by the P. Messing (1965) reported that applications of lime and P to an acid, well drained soil decreased the more easily available Mn levels, but increased the less available forms.

Table 17 shows a large decrease in acid-extractable and exchangeable Mn when the soil was put in the pots for treatments. As in Experiment III the fumigation with CH_3Br several years before caused large amounts of manganese oxides to be converted to Mn^{+2}. Kept dry while in storage, the Mn^{+2} was not converted back to the manganic oxides. When exposed to air and water, however, oxidation took place rapidly. If the Mn^{+2} level had remained high, toxicity would have been more likely.

The large amounts of exchangeable and acid-extractable Mn in the original soil samples indicated the soil was high in total Mn. Additionally, these levels were high at pH values where Mn toxicity would not generally be considered a problem. Their rapid decrease upon the soil being put in the pots and being watered indicated how reactive Mn ions were in the soil.

Besides fumigation and acidity, water-logging and compaction can also increase available soil Mn levels. A golf green is subject to all of these factors, especially compaction. Consider the possibilities if a golf green was built from this soil. It is a heavy soil with poor drainage and high Mn levels. Fumigation of the soil before greens construction would result in increased Mn availability. After construction, with a large number of golfers using the green, compaction would be severe. This would be especially true when mechanical aerification is not possible, and wet weather is prevalent. These reducing conditions favor the conversion of manganic oxides to Mn^{+2}. Because Mn ions are so unstable in the soil, this conversion could take place rapidly and toxic levels be quickly attained.

Plant Mn concentrations were measured to determine what effect lime and P had on them, and to compare them to plant Mn concentrations found in plants grown in nutrient solutions. Highest plant Mn concentrations occurred when no lime was applied (Table 18). Manganese concentrations were also reduced at the first harvest when P was applied at the highest rate of lime. Messing (1965) found in a high Mn acid soil,applications of P reduced the Mn content of lettuce plants. The pH of the soil increased either slightly or remained unchanged by the treatment.

A regression analysis indicated that plant Mn concentrations were more highly correlated with exchangeable soil Mn levels (r^2 = .96, first harvest; r^2 = .62, second harvest; Figure 8) than with Mn extracted with the weak acid (r^2 = .04, first and second harvests). This was an indication Mn in the soil readily available for plant uptake would

Table 18. Effect of applications of lime and P on the top Mn concentration of Penncross bentgrass grown on soil III at two harvest dates

Treatments		First Harvest	Second Harvest
Lime	P		
Kg/a		Mn (ppm)	
00.0	0.00	323b	404a
00.0	2.44	371a	442a
00.0	4.88	392a	437a
24.4	0.00	231d	335b
24.4	2.44	229d	353b
24.4	4.88	254cd	327bc
48.8	0.00	277c	292cd
48.8	2.44	232d	280d
48.8	4.88	194e	258d

Means within a column with a common letter are not significantly different at the 5% level according to Duncan's New Multiple Range Test.

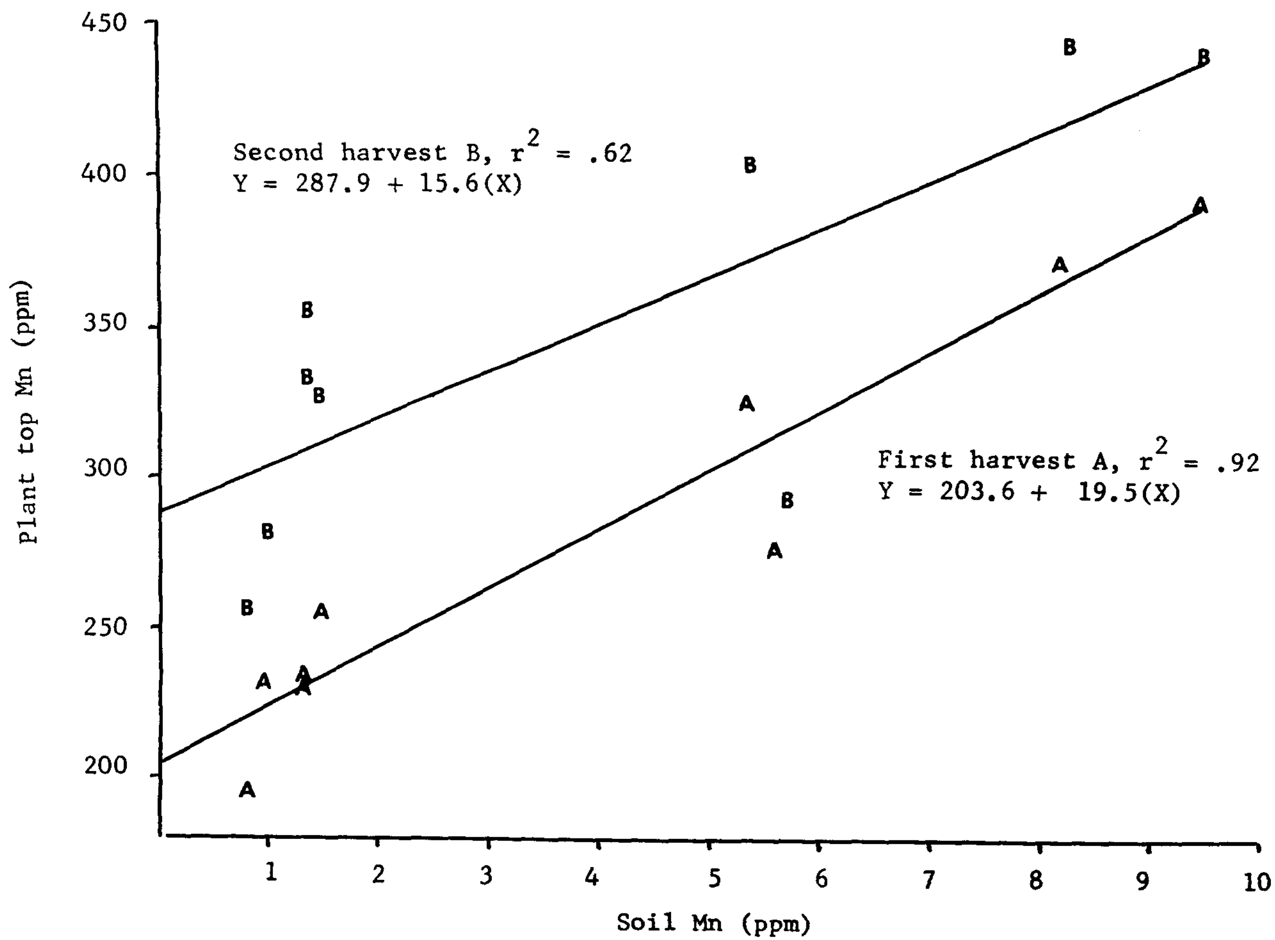

Figure 8. Relation between Mn concentration in tops of Penncross bentgrass and NH_4OAc extractable Mn in the soil, A = first harvest, B = second harvest

be a better indicator of toxicity in Penncross bentgrass than a measure of the potentially available Mn. However, since the pH of an acid extractant is also a factor in determining soil Mn levels, soil pH should also have been considered when performing the correlation.

The mean plant Mn concentrations at the first and second harvest were 278 and 347 ppm Mn respectively. Both means were closest to the Mn concentration in unclipped plants grown in nutrient solution containing 1.0 ppm Mn (307 ppm plant Mn). Only at the second harvest on treatments where lime was omitted did plant Mn concentrations equal those found in unclipped plants grown in nutrient solutions containing 2.0 ppm Mn. No plant Mn concentration approached the toxic level of approximately 2000 ppm Mn.

SUMMARY

Experiments were conducted to study the effect of varying concentrations of solution Mn on the growth of Penncross bentgrass. In addition, the growth of Penncross bentgrass on three golf green soil mixes high in Mn and the effect of lime and P on the availability of Mn in the soil mixes were evaluated.

The data from sand cultures indicated that 1.0 ppm Mn in solution was adequate for good plant growth. Initial appearance of toxic symptoms in the tops and roots of clipped plants occurred at 8.0 and 16.0 ppm Mn in solution respectively. Unclipped plants required four times the concentration of solution Mn to produce toxic symptoms similar to those of the clipped plants. The toxic concentration of Mn in the plant exceeded 7000 ppm in the roots and 2000 ppm in the tops.

Increasing concentrations of solution Mn decreased top growth in clipped plants, but not in the unclipped ones. Root weights increased in both clipped and unclipped plants with increases in solution Mn. This was probably caused by abnormal root swelling, increased cellular root material, and oxidation of the increased Mn content. As a result of the high Mn levels, uptake and translocation of Fe, Ca, and Mg from the roots to the tops decreased.

Manganese availability in the three golf green soil mixes were originally high due to artificial or unnatural treatment of the soil, i.e., storage conditions and methyl bromide fumigation. At the beginning of the experiment, these levels quickly decreased to

below the toxic level to the grass. If grown under golf green conditions, where reducing conditions are prevalent and plants are mowed daily, toxicity to the plants would have been more likely.

Applications of lime to the soil mixes appeared to increase acid-extractable Mn and decrease exchangeable Mn, whereas, P had little effect on either. Soil pH largely controlled exchangeable Mn levels, but had little effect on acid-extractable Mn. The data suggested that 24.4 kg/a of lime was the optimum rate to reduce Mn availability in the soil. Plant Mn concentrations correlated better with exchangeable Mn than with acid-extractable Mn when pH was not considered. Highest plant Mn concentrations in the bentgrass grown on the soil mix highest in Mn were only one-fifth that amount determined to be toxic to the grass in solution culture tests.

LIST OF REFERENCES

Adams, F. and I. I. Wear. 1957. Manganese toxicity and soil acidity in relation to crinkle leaf of cotton. Soil Sci. Soc. Amer. Proc. 21:305-308.

Anderson, Irvin and H. J. Evans. 1956. Effect of manganese and certain other metal cations on isocitric dehydrogenase and malic enzyme activities in Phaseolus vulgaris. Plant Physiol. 31:22-28.

Anderson, J. M. and N. A. Pyliotis. 1969. Studies with manganese deficient chloroplasts. Biochimica Et. Biophysica Acta. 189:280-293.

Andrews, C. S. and M. P. Hegarty. 1969. Comparative responses to manganese excess of eight tropical and four temperate pasture legume species. Aust. J. Agric. Res. 20:687-696.

Berger, K. C. and G. C. Gerloff. 1947. Manganese toxicity of potatoes in relation to strong soil acidity. Soil Sci. Soc. Amer. Proc. 12:310-314

Boken, Else. 1952. On the effect of storage and temperature on the exchangeable manganese in soil samples. Plant and Soil 4:154-163.

Bortner, C. E. 1935. Toxicity of manganese to Turkish tobacco in acid Kentucky soils. Soil Sci. 39:15-24.

Bradfield, R. L., R. Batjer, and J. Oskamp. 1934. Soils in relation to fruit growing. IV. The significance of the oxidation reduction potential in evaluating soils for orchard purposes. New York Agr. Exp. Sta. Bull. 592.

Brown, A. L., F. J. Hills, and B. A. Krantz. 1968. Lime, P, K, and Mn in sugar beets and sweet corn. Agron. J. 60:427-429.

Buol, S. W. 1974. Personal communication.

Cheng, B. T. and G. J. Ouellette. 1968. Effect of various anions on manganese toxicity in Solanum tuberosum. Can. J. Soil Sci. 48:109-115.

Cheniae, G. M. and I. F. Martin. 1970. Sites of function of manganese within photosystem II. Roles in O_2 evolution and system II. Biochemica Et. Biophysica Acta. 197:219-239.

Corey, R. B. and E. E. Schulte. 1973. Factors affecting the availability of nutrients to plants. p. 23-33. In L. M. Walsh and J. D. Beaton (ed.). Soil testing and plant analysis. Soil Sci. Soc. Amer., Inc. Madison, Wisconsin.

Cox, F. R. 1968. Development of a yield response prediction and and manganese soil test interpretation for soybeans. Agron. J. 60:521-524.

Doi, Y. 1952. Studies on the oxidizing power of roots of crop plants. 1. The difference of crop plants and wild grasses. 2. The variation between paddy rice and soybeans. Proc. Crop. Sci. Soc. Japan 21:12-13 and 14-15.

Epstein, E. and P. R. Stout. 1951. The micronutrient cations iron, manganese, zinc, and copper: their uptake by plants from the absorbed state. Soil Sci. 72:47-65.

Fergus, I. F. 1954. Manganese toxicity in an acid soil. Queensland J. Agr. Sci. 11:15-27.

Foy, C. D., A. L. Flemming, and W. H. Armiger. 1969. Differential tolerance of cotton varieties to excess manganese. Agron. J. 61:690-694.

Foy, C. D., A. L. Flemming, and J. W. Schwartz. 1973. Opposite aluminum and manganese tolerance of two wheat varieties. Agron. J. 65:123-126.

Fujimoto, C. K. and G. D. Sherman. 1945. The effect of drying, heating, and wetting on the level of exchangeable manganese in Hawaiian soils. Soil Sci. Soc. Amer. Proc. 10:107-112.

Gavalas, N. A. and H. E. Clark. 1971. On the role of manganese in photosynthesis. Kinetics of photoinhibition in manganese-deficient and 3-(4- chlorophenyl)-1, 1 - dimethylurea-inhibited Euglena gracilis. Plant Physiol. 47:139-143.

Geering, H. R., J. F. Hodgson, and Caroline Sdano. 1969. Micronutrient cation complexes in soil solution: IV. The chemical state of manganese in soil solution. Soil Sci. Soc. Amer. Proc. 33:81-85.

Gerloff, G. C., D. G. Moore, and J. T. Curtis. 1966. Selective absorption of mineral elements by native plants of Wisconsin. Plant and Soil 25:393-405.

Graven, E. H., O. J. Attoe, and Dale Smith. 1965. Effect of liming and flooding on manganese toxicity in alfalfa. Soil Sci. Soc. Amer. Proc. 29:702-706.

Hale, J. B. and S. G. Heintze. 1946. Manganese toxicity affecting crops on acid soils. Nature 157:554.

Hammes, J. K. and K. C. Berger. 1960. Chemical extraction and crop removal of manganese from air-dried and moist soils. Soil Sci. Soc. Amer. Proc. 24:361-364.

Heath, R. L. and G. Hind. 1969. On the functional site of manganese in photosynthetic oxygen evolution. Biochemica Et. Biophysica Acta. 189:222-233.

Heintze, S. G. 1946. Manganese deficiency in peas and other crops in relation to the availability of soil manganese. J. Agric. Sci. 36:227-238.

Heintze, S. G. and P. J. G. Mann. 1947. Soluble complexes of manganic manganese. J. Agric. Sci. 37:23-26.

Heintze, S. G. and P. J. G. Mann. 1949. Studies on soil manganese. II. The exchange properties of the manganese of neutral and alkaline organic soils. J. Agric. Sci. 39:87-95.

Hemstock, G. A. and P. F. Low. 1953. Mechanisms responsible for retention of manganese in the colloidal fraction of soil. Soil Sci. 76:331-343.

Hiatt, A. J. and J. L. Ragland. 1963. Manganese toxicity of burley tobacco. Agron. J. 55:47-49.

Hoff, D. J. and H. J. Mederski. 1958. The chemical estimation of plant available soil manganese. Soil Sci. Soc. Amer. Proc. 22:129-132.

Homann, P. H. 1967. Studies on the manganese of the chloroplast. Plant Physiol. 42:997-1007.

Hoyt, P. B. and Marvin Nyborg. 1971. Toxic metals in acid soil: II. Estimation of plant-available manganese. Soil Sci. Soc. Amer. Proc. 35:241-244.

Itoh, Michio, Katsuji Yamashita, Tsugio Nishi, Katsuko Konishi, and Kazuo Shibato. 1969. The site of manganese function in photosynthetic electron transport system. Biochimica Et. Biophysica Acta. 180:509-519.

Jones, L. H. P. 1957. a. The effect of liming a neutral soil on the uptake of manganese by plants. Plant and Soil 8:301-314.

Jones, L. H. P. 1957. b. The effect of liming a neutral soil on the cycle of manganese. Plant and Soil. 8:315-327.

Leeper, G. W., 1947. Forms and reactions of manganese in soil. Soil Sci. 63:79.

Lucas, R. E. and B. D. Knezek. 1972. Climatic and soil conditions promoting micronutrient deficiencies in plants. p. 265-288. In J. J. Mortvedt, P. M. Giordano, and W. L. Lindsay (ed.) Micronutrients in agriculture. Soil Sci. Soc. Amer., Inc. Madison, Wisconsin.

Mass, E. V., D. P. Moore, and B. J. Masor. 1969. Influence of calcium and magnesium on manganese absorption. Plant Physiol. 44:796-800.

Matocha, J. E. 1973. Influence of high manganese rates on the growth and manganese levels in Bermudagrasses. Agronomy Abstracts: p. 100.

Meek, B. D., A. J. MacKenzie, and L. B. Grass. 1968. Effect of organic matter, flooding time, and temperature on the dissolution of iron and manganese from soil *in situ*. Soil Sci. Soc. Amer. Proc. 32:634-638.

Messing, J. H. L. 1965. The effects of lime and superphosphate on manganese toxicity in steam-sterilized soil. Plant and Soil 23:1-16.

Morgan, P. W., H. E. Joham, and J. U. Amin. 1966. Effect of manganese toxicity on the indoleacetic acid oxidase system of cotton. Plant Physiol. 41:718-724.

Morris, H. D. 1948. The soluble manganese content of acid soils and its relation to the growth and manganese content of sweet clover and lespedeza. Soil Sci. Soc. Amer. Proc. 13:362-371.

Morris, H. D. and W. H. Pierre. 1947. The effect of calcium, phosphorous and iron on the tolerance of lespedeza of manganese toxicity in culture solutions. Soil Sci. Soc. Amer. Proc. 12:382-386.

Mulder, E. G., and F. C. Gerretsen. 1952. Soil manganese in relation to plant growth. Advance Agron. 4:221-277.

Neenan, M. 1960. The effect of soil acidity on the growth of cereals with particular reference to the differential reaction of varieties thereto. Plant and Soil 12:324-338.

Ouellette, G. J. 1952. The toxicity of manganese in strongly acidic soils. Chem. Abs. 46:4157.

Ouellette, G. J. and L. Dressureaux. 1958. Chemical composition of alfalfa as related to degree of tolerance to manganese and aluminum. Can. J. Plant Sci. 38:206-214.

Page, E. R., 1962. Studies in soil and plant manganese. II The relationship of soil pH to manganese availability. Plant and Soil 16:247-257.

Page, E. R., E. K. Schofield-Palmer, and A. J. McGregor. 1962. Studies in soil and plant manganese. I. Manganese in soil and its uptake by oats. Plant and Soil 16:238-246.

Petersen, P. J. 1969. The distribution of ^{65}Zn in Agrostis tenuis (Sibth.) and A. stolonifera tissues. J. Exp. Bot. 20:863-875.

Pitty, Alistair, F. 1971. Inter-relationships between processes and land forms. p. 172-326. In Geomorphology. Methuen and Co. Ltd.

Reikels, J. W., and J. C. Lingle. 1966. Iron uptake and translocation by tomato plants as influenced by root temperature and manganese nutrition. Plant Physiol. 41:1095-1101.

Rich, C. I. 1956. Manganese content of peanut leaves as related to soil factors. Soil Sci. 82:353-363.

Robie, R. A. and D. R. Waldbaum. 1968. Thermodynamic properties of minerals and related substances of 298.15^{o} K (25.0^{o}C) and one atmosphere (1.013 bars) pressure and at higher temperatures. Geol. Sur. Bull. 1259. U. S. Government Printing Office, Washington, D. C.

Robson, A. D., and J. F. Laneragan. 1970. Sensitivity of annual Medicago species to manganese toxicity as affected by calcium and pH. Aust. J. Agric. Res. 21:223-232.

Sanchez, Carlostadio, and E. J. Kamprath. 1959. Effect of liming and organic matter content on the availability of native and applied manganese. Soil Sci. Soc. Amer. Proc. 23:302-304.

Sartain, J. B. 1973. Personal Communication.

Schnehl, W. R., Michael Peech, and Richard Bradfield. 1950. Causes of poor growth of plants on acid soils and beneficial effects of liming: I. Evaluation of factors responsible for acid soil injury. Soil Sci. 70:393-410.

Scrutton, M. C., M. F. Utter, and A. S. Milduan. 1966. Pyruvate carboxylase. VI. The presence of tightly bound manganese. J. Biol. Chem. 241:3480-3487.

Shive, J. W. 1941. Significant roles of trace elements in the nutrition of plants. Plant Physiol. 16:435-445.

Smith, D. H. 1963. Effects of fumigants on the soil status and plant uptake of certain elements. Soil Sci. Soc. Am. Proc. 27:538-541.

Snyder, G. H., and G. J. Gascho. 1973. Manganese induced iron chlorosis in St. Augustine turf. Florida Turf 7:3.

Somers, I. J. and J. W. Shive. 1942. The iron-manganese relation in plant metabolism. Plant Physiol. 47:582-602.

Stonier, Tom, Felix Rodriguez-Tormes, and Yoshiaki Yoneda. 1968. Studies on auxin protectors. IV. The effect of manganese on auxin protector-I of the Japanese morninglory. Plant Physiol. 43:69-72.

Tanaka, A. and S. A. Navasero. 1966. Manganese content of the rice plant under water culture conditions. Soil Sci. and Plant Nutr. Vol. 12 No. 2.

Teicher-Zallen, Doris. 1969. The effect of manganese on chloroplast structure and photosynthetic ability of _Chlamydomonas reinhardi_. Plant Physiol. 44:701-710.

Tiller, K. G. 1963. Weathering and soil formation of dolerite in Tasmania with particular reference to several trace elements. Aust. J. Soil Res. 1:74-90.

Timonin, M. I. 1946. Microflora of the rhizosphere in relation to the maganese-deficiency disease of oats. Soil Sci. Soc. Amer. Proc. 11:284-292.

Tisdale, S. L. and W. L. Nelson. 1966. Sulphur and microelements in soils and fertilizers. p. 288-356. _In_ Soil Fertility and Fertilizers. The Macmillan Company.

Turner, R. G. 1969. a. Heavy metal tolerance in plants. p. 399-410. _In_ H. Rorison (ed.) Ecological aspects of the mineral nutrition of plants. Brit. Ecol. Soc. Symp. No. 9. Blackwell Scientific Publications, Oxford.

Turner, R. G. 1969. b. The subcellular distribution of zinc and copper within the roots of metal tolerant clones of _Agrostis tenuis_ sibth. New Phytol. 69:725-731.

Turner, R. G. and R. P. G. Gregory. 1967. The use of radioisotopes to investigate heavy metal tolerance in plants. p. 493-509. _In_ Isotopes in plant nutrition and physiology. IAEA, Vienna, Austria.

Weinstein, L. H. and W. R. Robbins. 1955. The effect of different iron and manganese nutrient levels on the catalase and cytochrome oxidase activities of green and albino sunflower leaf tissues. Plant Physiol. 30:27-32.

White, R. P. 1970. Effects of lime upon soil and plant manganese levels in an acid soil. Soil Sci. Soc. Amer. Proc. 34:625-629.

Williams, D. E. and James Vlamis. 1957. Manganese and boron toxicities in standard culture solutions. Soil Sci. Soc. Amer. Proc. 21:205-209.