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Zinc Uptake in Loblolly Pine Seedlings

Victor Zillmer Stephen F Austin State University

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Zinc Uptake in Loblolly Pine Seedlings

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ZINC UPTAKE IN LOBLOLLY PINE SEEDLINGS

BY

Victor B. Zillmer, 8.S.F.

Presented to the Faculty of the Graduate School of Stephen F. Austin State University In Partial Fulfillment

of the Requirements

For the Degree of Master of Science in Forestry

STEPHEN F. AUSTIN STATE UNIVERSITY

August, 1978

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TABLE OF CONTENTS

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LIST OF TABLES

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INTRODUCTION

In 1975, the South-wide Expanded Southern Pine Beetle Research and Applications Program (ESPBRAP) was initiated to find possible relationships between the southern pine beetle (Dendroctonus frontalis Zimm.), and site factors that might predispose a timber stand to attack. From a part of this study, conducted at the School of Forestry, Stephen F. Austin State University, a negative relationship was found between soil zinc concentration and southern pine beetle attacks. Analysis of wood core samples from beetle killed trees in the ESPBRAP program found the same highly significant negative relationship between zinc concentration in the stem and beetle infestation incidence.¹

The effect of zinc on the southern pine beetle may not be direct. The blue stain fungi (Ceratocystis minor [Hedgc.] Hunt) is invariably found with the beetle (Craighead, 1928) and, apparently is responsible for killing the tree by blocking the movement of water through the xylem (Nelson, 1930). Successful development of the beetle larvae is believed dependent on the lower moisture content of the wood after xylem blockage (Bramble and Holst, 1940).

In an experiment conducted in the School of Forestry, growth of blue stain fungi in potato dextrose agar medium was inhibited at agar zinc concentrations of 8 ppm and completely controlled at concen-

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¹Watterston, K. G., unpublished data, School of Forestry. Stephen F. Austin State University, Nacogdoches, Texas.

trations of 10 ppm or greater.² Wood concentrations of zinc were commonly found to be lower than 8 ppm with the average concentration for the beetle killed trees to be less than 5.4 ppm.³

This thesis study is part of a larger work dealing with the possibility of controlling the southern pine beetle through the inhibition of the blue stain fungi using zinc as a systemic fungicide. The purpose of this study is to determine the levels of zinc that can be obtained in the roots, stem, terminal growth, and needles of loblolly pine seedlings with various levels of zinc fertilizer. Although height growth was recorded, the emphasis was on obtaining equations that would predict zinc levels through zinc fertilization. Due to the possibility of zinc inhibiting the beneficial mycorrhizal fungi, treatment effects on this fungi were also observed.

 2 Palmer, H. A., and K. G. Watterston, unpublished manuscript, School of Forestry. Stephen F. Austin State University.

^{3&}lt;sub>Zillmer</sub>, V. B., and K. G. Watterston, unpublished data, School of Forestry. Stephen F. Austin State University.

LITERATURE REVIEW

The common mineral sources of zinc in the soil are zinc sulfate, (sphalerite, $ZnSO_A$), zinc carbonate, (smithsonite, $ZnCO_z$), and zinc silicate, (Hemiphorite, $\text{Zn}(OH)_{2}\text{Si}_{2}\text{O}_{7} \cdot H_{2}O$), with sphalerite considered to be the primary inorganic source (Krauskopf 1972). Secondary major sources of zinc are the organic complexes called chelates. In most soils, zinc will be available in both the ionic and chelated form. As with most other metallic micronutrients, ionic zinc is primarily available below pH 7. Chelated zinc is ten times more common than ionic zinc above pH 7. As the pH of the growing medium increases above 7, the level of available ionic zinc decreases until pH 9, where zinc will precipitate out as $Zn(OH)_2$. The chelated zinc form by contrast, has a peak availability when the conditions for microorganisms are best: warm, moist, well-aerated soils of neutral pH (Stevenson and Ardakani 1972).

Zinc mobility depends on the form, with chelated zinc having a higher rate of movement than ionic zinc; ionic zinc is quite immobile due to a strong double positive charge holding the ion to the negatively charged soil particles (Gilkes et al. 1975). Chelated zinc is the main source of zinc movement through the soil profile, due to the organic molecules reducing the total positive charge that holds the zinc to the soil matrix.

Zinc uptake by the plant is conducted by both metabolic (Bowen 1969), and nonrnetabolic processes (Gulknecht, 1961, 1963). Metabolic

3

uptake requires some response by the plant to environmental conditions and is characterized by an uptake level that is fairly constant over time. On the other hand, nonmetabolic uptake is a condition where uptake is proportional to the amount available in the growing medium. Such nonmetabolic uptake is characterized by a large increase in the amount of uptake after an application, with a steady decrease in the uptake rate as the level in the growing medium decreases.

Temperature and light intensity can effect the rate and type of zinc absorbed. Corn (Zea mays), under low growing temperatures, $(5^{\circ}C)$, tends to absorb more organic zinc than inorganic zinc, and increasing temperatures will reverse this condition (Gallager et al 1978). As temperature increases, corn will absorb more zinc, possibly due to increased root growth contacting new sources of soil zinc, in both acid and calcareous soils (Bauer and Lindsay 1965). Light effects zinc uptake, possibly through the effect of light on temperature. Cool spring conditions are caused, in part, by a decrease in solar radiation during the winter months, hence low light effect on zinc uptake may actually be the effect of temperature on zinc uptake. It is also possible that low light intensity could cause a drop in zinc uptake by causing a decrease in physiological activity.

It has been widely reported that zinc and phosphorus are antagonistic in that an application of phosphorus will precipitate zinc in the form of zinc phosphate, a less available form. Increasing soil moisture will decrease the availability of zinc (Armeanu 1971) possibly due to the increase availability of phosphorus at increasing moisture levels. Phosphorus fertilization was found to reduce the amount of

 $\sqrt{4}$

zinc in the needles of Douglas fir growing on zinc polluted soils in the Netherlands (Van der Berrg et al 1973). An increase in either phosphorus or zinc will decrease the content of the other element available to rice (Oryza sativa) (Tiwari and Pathok 1976).

A few studies show an antagonism between zinc and iron. Iron or zinc application to rice will cause a decrease in the uptake of the other element (Subrahmanyam and Mehra 1974). This relationship could be caused by like positive charges competing for the same site. High zinc uptake in corn will decrease root and shoot fresh weight, and an application of iron will reverse the problem (Rosen et al 1977).

Zinc in plant tissues is important in the formation of RNA and ribosomes, which function in the control of cellular activity and protein synthesis respectively (Brown et al 1968). Shoog (1940) found a zinc deficiency resembles an auxin deficiency: frenching, rossettes, chlorosis, and dieback. A zinc deficiency could develop on acid, leached, sandy soils of non-zinc bearing mineralogy (Chapman 1966). Zinc levels for normal growth and development range from 16 to 30 ppm in walnut (Juglands nigra L.), 6 to 43 ppm in peach (Prunus persica L.), and 15 to 35 ppm in tung (Aleurites fordi), for foliage. Zinc levels for normal growth and development in stems of walnut are 24 to 34 ppm, ahd 11 to 55 ppm in peach. Zinc deficiency levels in the foliage of walnut are 11 to *2L* ppm, of tung are 3 to 6.2 ppm and of peach are 6 to 15 ppm. Deficiency levels for the stem of walnut are 8 to 17 ppm, and 5 to 12 ppm in peach.

5

(Chandler 1933), (Gaddum et al. 1936). Zinc deficiency in pecan (Carya illinoensis Wang.) has been found when total zinc level in soil is between 58 and 68 ppm (Alben and Boggs 1936). Lynan and Dean (1942), using ammonium acetate at pH 6, found available zinc levels of 0.5 to 0.6 ppm in soils where pineapple (Ananas comosas) had severe zinc deficiency, and 1.7 to 3.5 ppm where no deficiency was observed.

Zinc toxicities can be observed, usually in the form of iron chlorosis, where the soil is polluted from lead mine dumps, some acid peats, soils from zinc mining sites, and soils with high zinc parent material (Chapman 1966). Toxic zinc level in orange (Citrus siensis) is 200 ppm, and 485 ppm in tung (Chapman 1960, Shear 1958).

PROCEDURE

One year old loblolly pine seedlings from the Indian Mound Nursery, Alto, Texas, were graded with large and small seedlings discarded. The remaining seedlings were planted in January of 1978. There were three seedlings to a 25 em plastic pot, with four pots per treatment in acid washed and distilled water leached sand as the growing medium (Table 1). Medium size seedlings from the same bundle were analyzed for their nutrient content (Table 2). The experiment was established in a new aluminum greenhouse, (with no other experiments being conducted) and watered with distilled water until the seedlings broke dormancy. Just before the seedlings broke dormancy, each pot received liquid formula fertilizer, 12-12-12.

Three experiments were conducted, the first group receiving treatment on March 15, with the seedlings being lifted on April 13. The second group was started on April 24, and ended on May 22. The third group started on June 10 , going through July 7, when the seedlings were lifted. Each pot was treated with 500 ml of a zinc nitrate solution at one of the following zinc concentrations; 0, (control), 0.5 ppm, 1 ppm, 5 ppm, 10 ppm, and 20 ppm. During the experiment, the seedlings were watered with distilled water at a rate so that water was never allowed to drain out of the bottom of the pot. On the same day, the height and diameter of each seedling of that group was measured in millimeters by placing a block of wood across the top of the pot and measuring from the top of the block to the tip of the

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Table 1. Nutrient content of randomly selected samples of acid washed sand after leaching with distilled water using an ammonium acetate extracting solution at pH 9.

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Table 2. Average nutrient content of some loblolly pine seedlings from the same seedling bundle as the planted seedlings.

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terminal bud to determine height. Diameter of the stem at the height of the block was determined with a micrometer in inches and then converted to millimeters. After four weeks, one seedling per pot was removed and placed on a table and grouped according to the mycorrhizae present as follows: none, very low, low, moderate, high, and very high.

After the mycorrhizae level had been determined, the seedlings were dried at 60°C for 24 hours, with each seedling being dried in a separate container. After drying, the seedlings were separated into the roots, stem, terminal growth, and needles. Each part was weighed, and then ashed for 8 hours at 480°C. The ash was dissolved in five drops of 10% Hel and diluted to 25 ml with distilled water. Zinc content of each seedling part was then determined on a Jarrell-Ash Atomic Absorption Spectrophotometer, using a 5 ppm standard.

A sample of sand was taken from each pot after the seedling had been removed and analyzed for zinc using a ln NH₄OAc extraction solution at pH 6.9 (USDA 1972). The extract was measured on an atomic absorption unit using a 5 ppm standard. The nutrient content of the sand before the treatments started was calculated using the same ammonium acetate extraction. Nitrogen content was determined by the Kjeldahl Method. Phosphorus was determined by the molybdate blue method (USDA 1972).

The following linear regression models were used to examine the relationship between the zinc treatments and measured variables:

$$
Y = b_0 + b_1(x)
$$

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$$
Y = b_0 + b_1(x_1) + \dots + b_n(x_n)
$$

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$$
Y = b_0 + b_1(x_1) + b_2(x_1^2)
$$

where Y is the predicted value or the dependent variable, b_0 is the Y intercept, and b_1 , $b_2 \ldots b_n$ are the partial regression coefficients for each independent variable x. The first equation is for a straight line, the second is for a response surface, and the third equation is for a curve linear relationship. The slope intercept is usually an indication of the amount of zinc that can be expected without the effects of treatment, soil zinc, or mycorrhizae. Large slope intercepts indicate sources of zinc other than those measured are contributing to the zinc level, while smaller intercepts can indicate that the measured variables supply most of zinc the plant has acquired. The larger the b_1 , ... b_n regression coefficients the greater the effect of that variable on the predicted value. Zinc treatment, residual soil zinc, and mycorrhizae were the three variables used to predict the dependent variables: stem zinc, root zinc, terminal growth zinc, leaf zinc, residual soil zinc, mycorrhize formation, height and diameter growth. Treatment and residual soil zinc were considered against mycorrhizae, to determine if zinc might have an inhibitive effect on the development of this fungi. The relationship of leaf zinc concentration and zinc concentration in the other plant parts was also studied to see if leaf zinc concentrations is ^a good indicator of the zinc status of the whole plant.

11

RESULTS AND DISCUSSION

Zinc uptake, both in concentration and total content, decreased with age of seedling, being highest in group 1, and lowest in the third group (Table 3). At the same time that zinc uptake was decreasing, height growth of the seedlings also decreased (Table 4). Since zinc is necessary for auxin and RHA activity, as growth decreases, zinc uptake would also be expected to decrease. Group 1 seedlings (March 15- April 13) has the greatest height growth, due to the first flush of growth falling in that time period. With some of the seedlings starting and terminating height growth at different times, it could be expected that height growth would not correlate strongly with any treatment effect, unless the seedling was put under or relieved from some type of stress. Zinc deficiency or toxicity symptoms never appeared and zinc treatment had little effect on height growth. The lack of correlation between height growth and zinc levels may be due to the way height growth was measured. Since the measurement was from the tip of the terminal bud at the beginning of the experiment to the tip of the terminal bud at the end of the experiment, those plants with two or more terminal shoots would not have the same increase in millimeters of growth as a plant with a single terminal, even though both plants could have the same amount of growth on a weight basis. The nitrate in the zinc nitrate fertilizer was in small enough quantities to not have any great effect on height growth.

The average zinc concentration in the different plant parts can

12

Table 3. Average zinc levels of different parts of loblolly pine seedling four weeks after being fertilized during different times of the growing season.

Average zinc content in milligrams in each plant part

Average zinc concentration in parts per million

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Table 5. Average zinc concentration in different parts of loblolly pine seedlings 28 days after treatment with various levels of zinc.

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be found in Table 5. The zinc concentrations of the April 24 - May 22 (group 2), are half of the March 15 - April 13 (group 1). The third group, June 10 - July 7, had an average zinc concentration of only about 40% of the second group. Since plants generally absorb more nutrients under growth conditions, as the growth rate increases, the uptake of zinc would also increase (Tables 4 and 5).

Of all regressions tested for significance, only the simple linear regression,

$$
Y = b_0 + b_1(x)
$$

and multiple regression,

$$
Y = b_0 + b_1(x_1) + \ldots b_n(x_n)
$$

were found to be able to predict zinc concentration based on Pearson correlation coefficient, ^r (Snedecor 1946). 'r' values describe how well an equation for ^a line fits ^a set of dependent and independent variables. 'r' values range from 1.0 to 0, with 1.0 being ^a perfect correlation, and zero showing no correlation. 'r' values can also be negative, from -1.0 to 0, denoting a negative correlation. Coefficients of determination, or r^2 , can be determined by squaring the correlation coefficient, r, and are used to show the amount of variation in the dependent variable y that is explained by a variation in the independent variable x. As an example, the formula:

$$
Y = 14.7457 + 1.3226 (x) \qquad r = .7440
$$

can be used to predict the zinc concentration in the root of loblolly pine seedlings, Y, when fertilized on March 15, from the zinc fertilization rate, (x). The slope intercept, 14.7457, indicates that when there is no zinc fertilization, there will be 14.7457 ppm

of zinc in the root. The slope factor, 1.3226, states that for each unit increase of zinc fertilizer, there will be a 1.3226 unit increase in zinc in the root. **'r'** values like .7440 are considered acceptable for biological studies, based on the number of observations, larger samples being significant at lower r values. The coefficient of determination, r^2 , for this problem is .5535 meaning that 55.35% of the variation of the dependent variable, root zinc concentration, is explained by the variation in the independent variable zinc fertilization rate.

Table 6 contains the average zinc concentration of the sand for each seedling, four weeks after the treatment. Table 7 contains linear regression equations derived from the residual soil zinc for each group. Both of these tables support the results in Tables 3 and ⁵ in that zinc uptake was the highest in the first group, March ¹⁵ - April 13. The poor r values can be explained by the presence of zinc in the sand after acid washing, and the application rates, on a soil weight basis, being less than 2 ppm. A correlation matrix for zinc concentrations in the different plant parts can be found in Table 8. From this table, various observations can be made about zinc uptake. Group 1 r values are the highest on the average, closely followed by group 3, with group 2 considerably lower. This phenomenon could be explained by seedlings being in active growth in group 1 and generally in slow growth in group 3, with group 2 having some seedlings that were actively growing and some that were not. The correlation between zinc treatment and root zinc increased with time, possibly due to less zinc being translocated out of the roots, giving a stronger relationship in

17

 \mathcal{L}_{max}

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Table 6. Average soil zinc extractable by ammonium acetate of pH 6.9 four weeks after various levels of zinc fertilization.

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Table 7. Prediction of soil zinc concentration (Y), from zinc

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Table 8. Correlation matrix for treatment, residual soil zinc, root zinc, stem zinc, terminal zinc, and leaf zinc for all three groups.

(1) March 15 - April 13

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(2) April 24 - May 22

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(3) June 10 - July 7

the later treatments that had less growth. Correlation of zinc treatment and terminal growth zinc concentration was the highest in group 1, and the least in group 2, also possibly effected by the greater uniformity of growth in groups 1 and 3. Leaf zinc correlated the highest in group 3, with the lowest in group 1.

Residual soil zinc correlated with root zinc the best in group 3, as did zinc treatment, although the correlations were lower. Stem zinc concentration correlated highly with residual soil zinc concentration in group 1, and an equation for this relationship can be found in Table 9. The relationship of residual soil zinc to terminal growth zinc is similar to that of terminal growth zinc and zinc treatment, highest in group 1 and lowest in group 2, and an equation expressing this relationship found in group 1 can be found in Table 9. Correlation of residual soil zinc and leaf zinc did not react in the same manner as zinc treatment and leaf zinc, for residual soil zinc correlated highest in group 1 and lowest in group 2, with an equation expressing the relationship found in group 1 in Table 9.

The relationships between the concentrations of zinc in the different plant parts can also be found in Table 8. In every single case, group 2 seedlings had the lowest relationships, probably due to the lack of uniformity of growth found in the other two groups. In four out of six cases, root to stem, root to terminal growth, root to leaf, and stem to terminal growth, group 3 had the highest correlation of zinc concentration between different plant parts, with group 1 having the two highest correlations of any found in this study, stem to leaf and terminal growth to leaf.

21

Table 9. Prediction of zinc concentration in the stem (sz), terminal growth (tz), root (rz), and leaf (lz), from zinc fertilization rates (trt), and residual soil zinc (rsd).

March 14 - April 13 (Group 1) $sz = 32.0475 + 38.4046(rsd)$ $r = .7805$ $tz = 24.6015 + 46.1558(rsd)$ $4 = .8170$ $1z = 1.8740 + 69.2904(rsd)$ $r = .7746$ $1z = 2.2840 - 1.6291 (trt) + 85.6635 (rsd)$ $r = .7867$ June 10 - July 7 (Group 3) $rz = 14.7457 + 1.3226(trt)$ $r = .7440$

Mycorrhizae were analyzed both as to the effect of zinc on mycorrhizae and the effect of mycorrhizae on zinc uptake. From the methods employed, it can only be determined that mycorrhizae had no effect on zinc uptake (Table 10), but it is possible that application of zinc in concentrations of 1 to 5 ppm can increase mycorrhizae formation (Table 11).

Zinc determination in plant tissue would be facilitated by not having to destroy the entire plant. To this end, linear regression equations have been developed to predict the zinc concentration in the stem, terminal growth, and roots from a leaf sample (Table 12). The equations show the same relationship as the raw data, that is, a higher zinc uptake level would be expected in the early spring, and lower levels of uptake when zinc is applied at a later date.

23

Table 10. Correlation coefficients of mycorrhizae, height growth, and diameter growth when regressed against treatment, residual soil zinc, and mycorrhizae.

All values are not significant

 $\mathcal{L}^{\text{max}}_{\text{max}}$

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 $\sim 10^{-10}$

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 (1) None = 0, Very Low = 1, Low = 2, Moderate Very High = $5.$ $3,$ High = 4,

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 $\sim 10^7$

Table 12. Prediction of stem, root, and terminal growth zinc concentration from leaf zinc concentration.

CONCLUSIONS

It is possible to predict the zinc concentration in the stem, terminal growth, roots, and needles of loblolly pine seedlings, grown in acid washed sand, from zinc fertilization, in the form of zinc nitrate, using linear and multiple regressions. Those seedlings that were treated during the first flush of growth absorbed more zinc than those which were fertilized later. Zinc concentration in the soil, after the treatments were ended, was positively correlated with zinc levels in the plant in some cases. Zinc levels in the needles of loblolly pine seedlings can predict the zinc levels in the stem, roots, and terminal growth, eliminating the need for total destruction of the plant in future studies. Neither zinc fertilization or residual soil zinc had any effect on mycorrhizal formation. Mycorrhizal formation had no effect on zinc uptake. Zinc fertilization, residual soil zinc, and mycorrhizae had no effect on height or diameter growth.

In most fertilization studies, a curvilinear relationship was found between treatment and growth or nutrient concentration. The linear relationships in this study demonstrate that the zinc fertilization levels were too low to be representative of the entire positive spectrum of zinc fertilizer effects on plant tissue zinc levels. In future studies, the zinc fertilization rate would have to be increased if the information on the critical concentrations and optimum rates are needed. Another area for study would be to determine the effects of secondary growth flushes and of zinc levels obtainable in the

27

tissue when loblolly pine seedlings are fertilized during dormancy. It would also be of interest to find the zinc levels obtainable in the stem of semimature loblolly pine. Also, it may be better to determine height growth on a weight basis than by measuring millimeter increase in the terminal growth.

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APPENDIX

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ZINC UPTAKE IN LOBLOLLY PINE SEEDLINGS

An Abstract of a Thesis

APPROVED:

Denmeth T. Wallach

Solen W. Joston Jr

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ZINC UPTAKE IN LOBLOLLY PINE SEEDLINGS

by

Victor B. Zillmer, B.S.F.

Presented to the Faculty of the Graduate School of Stephen F. Austin State University In Partial Fulfillment of the Requirements

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For the Degree of Master of Science in Forestry $\bar{\mathbf{v}}$

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STEPHEN F. AUSTIN STATE UNIVERSITY

August, 1978

ABSTRACT

Zinc concentrations in the root, terminal growth, stem, and needles of loblolly pine (Pinus taeda L.) were measured in 72 seedlings grown in washed sand, fertilized with $\text{Zn}(N0_3)_2$. Zinc treatment periods were early spring, late spring, and summer. Zinc uptake was the greatest in early spring. Zinc nitrate fertilizer and remaining soil zinc at treatments end had no effect on mycorrhizae, height, or diameter growth. Linear and multiple regressions were derived using residual soil zinc and treatment zinc that can predict zinc concentrations in different plant parts that have r values from .7440 to .8170. Linear regressions for the prediction of zinc concentrations in different plant parts from leaf zinc concentration have r values from .7694 to .9146.

VITA

Victor Zillmer was born in Larned, Kansas, on April 11, 1955, son of Dr. H. Lawrence and Mary Louise Zillmer. After completing his work at Nacogdoches High School, Nacogdoches, Texas, in 1973, he entered Stephen F. Austin State University at Nacogdoches, Texas. He received his Bachelor of Science in.Forestry from Stephen F. Austin State University in August 1977. In September, 1977, he entered the Graduate School of Stephen F. Austin State University.

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