

Figure 1. Stunted growth and short needles are two symptoms of zinc (Zn) deficiency in pine seedlings. The container on the right contains normal loblolly pine seedlings growing in a nutrient solution containing zinc chloride (ZnCl₂) (Lyle 1969). The seedlings on the left were grown in a nutrient solution that did not contain Zn. Deficient seedlings had short, thick, and twisted secondary needles. Photo by Jack May.



Land Leveling Can Cause Temporary Zinc-Deficiency in Pine Seedlings

David B. South

Emeritus Professor, School of Forestry, Wildlife and Environmental Sciences, Auburn University, AL

Abstract

Land leveling can cause zinc (Zn) deficiencies in corn (*Zea mays* L.) by removing topsoil and lowering the population of endomycorrhizal spores. Although Zn deficiencies on pine (*Pinus* spp.) seedlings are rare in established bareroot nurseries where ectomycorrhizal spores are airborne, a brief deficiency occurred after leveling “new ground” in Alabama. In July 1986, nonmycorrhizal loblolly pine (*Pinus taeda* L.) seedlings had purple cotyledons and short needles when planted on newly leveled ground. Plots of stunted seedlings were sprayed with a phosphorus (P) fertilizer, which increased height growth. The Zn deficiency lasted less than 7 months. In August, foliage on stunted seedlings in untreated plots ranged from 4 to 11 ppm Zn, but in November the level exceeded 90 ppm. The risk of a Zn or P deficiency in pine seedlings is low when short roots are ectomycorrhizal. When P and Zn deficiencies occurred on nonmycorrhizal loblolly pine seedlings, however, the Zn deficiency was overlooked since stunting and short needles are symptoms of both deficiencies.

Introduction

Although zinc (Zn) deficiencies in endomycorrhizal crops might be the most ubiquitous micronutrient deficiency worldwide (Alloway 2008, Swietlik 1999), Zn deficiencies in bareroot pine (*Pinus* spp.) seedlings are rare. Zn deficiencies have been reported on an 88 percent peat soil in New Zealand (Knight 1976) and at a nursery in Wisconsin (Tanaka et al. 1967). Although stunted pines at a Colorado nursery had foliage with 11 ppm Zn, stunted growth was likely due to alkaline soil with high soil calcium (Ca) (Landis 1988). Furthermore, pine seedlings with 9 ppm foliar Zn were classified as healthy (Knight 1976).

Zn-deficient pine seedlings can be grown in greenhouses under controlled conditions (Lyle 1969, Smith and Bayliss 1942), although Zn deficiency symptoms do not always appear (Hacskeylo et al. 1969). Two common symptoms on loblolly pine (*Pinus taeda* L.) are stunting and short needles (figure 1). In some species, Zn-deficient seedlings have a dark-green color (figure 2). Chlorotic needles are not a typical symptom of Zn deficiency in pines.

When preparing land for a new pine nursery, topsoil is often removed and stockpiled before land-leveling operations (Morby 1984). When leveling is completed,

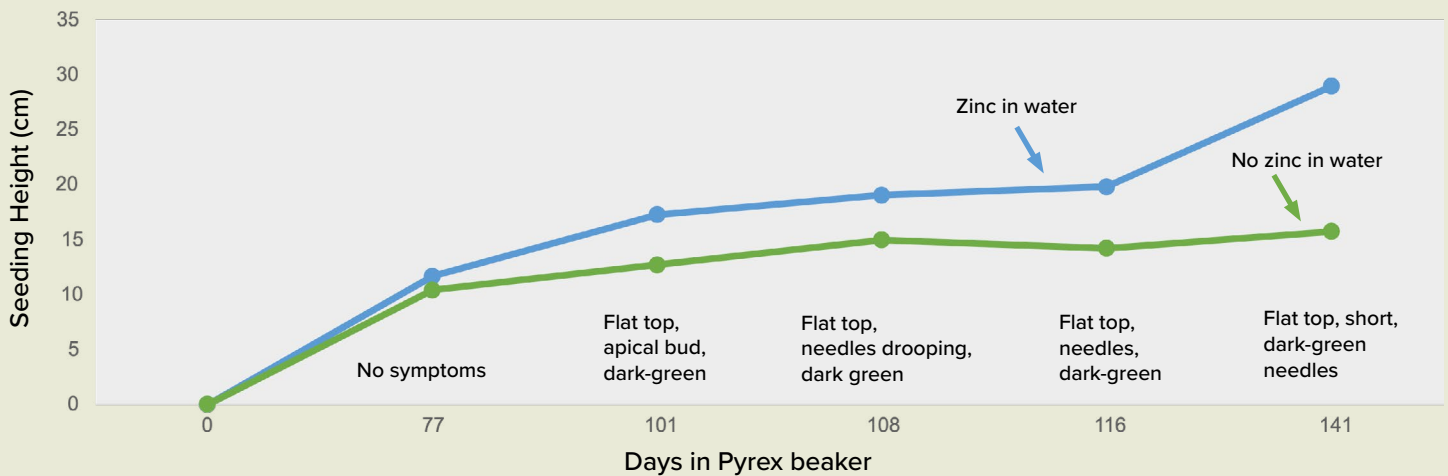


Figure 2. When growing in aerated water with no zinc (green line), Monterey pine seedlings show deficiency symptoms (stunting, dark-green needles, and short needles) between weeks 11 and 15. Adapted from Smith and Bayliss (1942).

topsoil is replaced, but the stockpiled soil may not be sufficient to cover all areas. Some spots may have 1 ppm Zn in soil (Grunes et al. 1961), while others may have more than 6 ppm (using the Mehlich 3 soil test). After land leveling, Zn deficiencies have occurred on several crops (Alloway 2008, Brye et al. 2004, Grunes et al. 1961, Shapiro 2019, Swietlik 1999, Viets et al. 1953). Land leveling combined with soil fumigation can deplete ectomycorrhizal spores and produce phosphorus (P) deficiencies (Trappe and Strand 1969), while land leveling alone can produce Zn deficiencies.

Determination of Zn Deficiency

Various methods are used to define Zn deficiency. The preferred way involves hypothesis testing with a fertilizer trial. For example, comparisons can be made among seedlings treated with zinc chloride ($ZnCl_2$), chelated Zn fertilizer, and untreated seedlings. If both Zn groups grow faster than the untreated group, then seedlings were likely Zn deficient. Another approach is to treat a group of seedlings with zinc sulfate ($ZnSO_4$) and assume that any growth response is due to the Zn supplement and not the sulfur (S) (i.e., seedlings were Zn, not S, deficient) (McKee 1976, Tanaka et al. 1967). A third method assumes Zn deficiencies can be determined simply by analyzing and comparing Zn levels in relation to foliage biomass. This math-based method is not scientific, but it is often used instead of hypothesis testing.

Seedlings with 9 ppm Zn in foliage can be considered normal (Knight 1976). This value is the threshold level used in this article. Defining deficient seedlings by comparing a foliar Zn test with a foliar distribution curve for Zn is not a

valid method. When asymptomatic pines have 11 to 30 ppm Zn in foliage, they are not Zn deficient (Jokela et al. 1991, Knight 1976, Ruiter 1969, Stone 1968).

Zn Deficiency Symptoms

Zn deficiency symptoms can be produced in hydroponic systems when the water contains little or no Zn. Deficiency symptoms include stunting, short needles, rosette buds, and dark-green or bronze needles. When grown in water, Zn-deficient loblolly pine seedlings appear stunted with short needles (see figure 1), while Monterey pine (*Pinus radiata* D. Don) seedlings are stunted with short, dark-green needles (figure 2). When growing in over-limed peat, pine seedlings develop a rosette of buds in place of the usual single bud and may exhibit bronze foliage (figure 3). Stunting and rosette buds were observed in pine plantations in Australia (McGrath and Robson 1984).

Sometimes Zn deficiency in conifers remains undiagnosed, especially in the past prior to routine micronutrient analyses. For example, following land leveling, soil fumigation resulted in stunted, nonmycorrhizal Douglas-fir (*Pseudotsuga menziesii* Mirb. Franco) seedlings (Trappe and Strand 1969), but the Zn status was unknown.

At a nursery in Alabama, P-deficient pine seedlings (figure 4) were diagnosed in July 1986 while Zn deficiency symptoms were overlooked. Since stunting and short needles are also symptoms of P deficiency (Haesckaylo et al. 1969, Lyle 1969), researchers concentrated on improving growth by treating seedlings with phosphoric acid. Decades later, however, the data files were reexamined and the Zn deficiency was discovered.

Materials and Methods

A fertilizer trial was established in 1986 at the Union Camp Nursery near Inverness, AL (32°06' N, 85°43' W, altitude 140 m). The study tested the hypothesis that P fertilization does not affect growth of stunted pine seedlings. The climate for this area is warm and humid with a mean annual precipitation of 1,288 mm. Prior to expanding the nursery, the study area supported a 23-year-old pine plantation, and timber was harvested during the summer of 1984. Stump removal began in August after which the soil was leveled. On July 15, 1985, millet (*Panicum ramosum* L.) was sown as a cover crop. The soil was fumigated (March 15, 1986) with methyl bromide-chloropicrin (448 kg/ha). Before sowing loblolly pine seed, the soil received 224 kg/ha of triple superphosphate (TSP) and 112 kg/ha of potassium chloride (KCl). Seeds were treated prior to sowing with the fungicide triadimefon at 12.5 g of active ingredient per 10 kg of seed.

Seeds were sown with a vacuum precision machine on April 9, and then oxyfluorfen was applied at 0.56 kg ai/ha. Postemergence applications of oxyfluorfen at 0.56 kg ai/ha were applied on June 13 and August 15. To control emerged grasses, sethoxydim was applied on June 13 and July 9. Foliar applications of triadimefon (0.14 kg ai/ha) were applied on May 5, May 12, and June 5 to control fusiform rust, caused by *Cronartium quercuum* (Berk.) Miyabe ex Shirae f. sp. *fusiforme* Birdsall and Snow. Ammonium nitrate was applied (100 kg/ha) on May 21 and July 8 and ammonium sulfate was applied (168 kg/ha) on June 12 and July 23. Rain in February (107 mm) and March (185 mm) was above normal, but rainfalls in April (18 mm), May (69 mm), and June (71 mm) were 98 mm, 34 mm, and 39 mm below normal, respectively (NOAA 1987).

Although germination was good, seedlings growing in the new, land-leveled ground showed signs of mosaic stunting in July (figure 4). On a few scattered areas, there were patches of normal, mycorrhizal seedlings.

Figure 3. Monterey pine seedlings from the Sweetwater Nursery in New Zealand showed a normal appearance (left) with 15 to 124 ppm foliar zinc (Zn) while stunted seedlings (to the right) had 1 to 5 ppm Zn (Knight 1976). Courtesy of Scion New Zealand, photo by H. Hemming, August 1974.



Figure 4. Stunted growth of loblolly pine seedlings was apparent during the first week of July 1986 in a recently land-leveled field at the Union Camp Nursery near Inverness, AL. Many seedlings were nonmycorrhizal, but a few patches of mycorrhizal seedlings were scattered throughout the field. Photo by David South, 1986.

had purple cotyledons, though most primary needles were short and green (figure 5). This condition may occur when reinoculation with airborne ectomycorrhizal spores is delayed due to inadequate soil moisture in adjacent forests.

Since stunted seedlings lacked mycorrhiza, a P fertilization study was established on July 29, 1986. Two treatments (phosphoric acid fertilization and a control) were replicated five times on an area with uniformly stunted seedlings. Each plot was 1.2 m long by 1 m wide. The P treatment involved spraying a 3-percent solution (w/w) of phosphoric acid (H_3PO_4) at a rate of 18.3 g of P per m^2 . Three weeks after treatment, seedlings were sampled for height, shoot dry weight, and root dry weight. Foliar samples were taken from each plot on August 18 (figure 6). On November 23, foliage and soil samples were collected for analysis at a laboratory in Memphis, TN.

Results

The phosphoric acid treatment increased growth and foliar P concentrations. Three weeks after treatment, acid-treated seedlings had 50 percent more mass and were 11 mm taller than untreated seedlings. By November, treated seedlings had twice as much mass and were 44 mm taller than untreated seedlings (South et al. 1988). Foliage of treated seedlings contained more P and sulfur (S) than nontreated seedlings in August, but this difference was



Figure 5. Stunted growth and short needles are two symptoms of phosphorus (P) deficiency in pine seedlings. This photo (July 29, 1986) compares stunted, nonmycorrhizal loblolly pine seedlings (left) with mycorrhizal seedlings (right). Stunted seedlings had a shoot dry mass of 173 mg while normal seedlings had a shoot dry mass of 576 mg (South et al. 1988). It is possible that P-deficient seedlings were also deficient in zinc (Zn). Foliar samples from five plots contained 5, 4, 7, 5, and 4 ppm Zn in August, and repeated samples collected in November contained 32, 25, 69, 73, and 34 ppm Zn, respectively. Photo by David South, 1986.



Figure 6. Stunted loblolly pine seedlings had short needles with purple cotyledons and 840 ppm foliar phosphorus. Seedlings on the right responded to a foliar-soil application of phosphoric acid (applied July 29). Photo by David South, 1986.

gone by November (table 1). Foliar Zn levels of 5 samples were below 9 ppm in August (4 to 7 ppm), but foliar samples from all 10 study plots ranged between 25 to 190 ppm by November. Soil samples collected in November indicated the acid treatment increased readily available P, manganese (Mn), and iron (Fe).

Discussion

Soil fumigation can kill mycorrhizal spores, but the risk of nonmycorrhizal roots increases on new ground that had topsoil removed prior to fumigation. Soil fumigated in the fall is typically reinoculated with airborne spores by April. For this reason, nursery managers should consider fumigating in the fall (Enebak et al. 1990, Hansen et al. 1990, Molina and Trappe 1984). During dry periods, production of ectomycorrhizal spores can be inadequate. At some nurseries, adding spores after fumigation increased the number of mycorrhizal roots (Marx et al. 1979, South 2018, Trappe and Strand 1969). At the Union Camp Nursery in 1986, natural inoculation with airborne spores was delayed due to low rainfall and apparently peaked between September and November. When soil fumigation delays mycorrhizal formation, seedlings can develop a P deficiency (South et al. 2018). In contrast, fumigation does not necessarily cause Zn deficiency. Under conditions of adequate available Zn, nonmycorrhizal pine roots can take up adequate Zn (Fomina et al. 2006, Hartley-Whitaker et al. 2000, Schier and McQuattie 1995). When Zn is inadequate due to land

leveling, however, a temporary deficiency can occur on nonmycorrhizal seedlings before the fall equinox. Since P deficiency symptoms include stunting and purple needles, stunting due to a Zn deficiency was overlooked. Even without soil fumigation, land leveling can result in stunted, endomycorrhizal crops (Grunes et al. 1961, Swietlik 1999).

Several reasons could explain the ephemeral nature (<17 weeks) of the Zn deficiency. First, as roots grow, they uptake Zn from the soil solution. Second, after mycorrhizae develop (July to November), Zn uptake increases as mycelia spread throughout the soil. Third, since groundwater contains Zn, irrigation during the growing season adds Zn to seedbeds. For example, when water contains 0.04 ppm Zn, 500 mm of irrigation will provide 0.2 kg/ha of Zn.

When foliage samples are taken too late in the growing season, reasons for stunted pine seedlings may go unexplained. Since foliar samples in November had 2,100 ppm P, some might claim the mosaic stunting in July (see figure 4) was not caused by nonmycorrhizal seedlings. Likewise, others may say there was no proof Zn was inadequate in seedlings sampled in July. Although stunted Monterey pine seedlings had foliage with 2 to 5 ppm Zn (Knight 1976), this is not proof that loblolly pine seedlings with 4 or 5 ppm Zn were stunted due to a Zn deficiency. For example, loblolly pine tolerates low Zn levels better than shortleaf pine (*Pinus echinata* Mill.) (Wilson 1953). Treatment of stunted pine seedlings with $ZnCl_2$ (instead of phosphoric acid) would show if the stunted seedlings were deficient in Zn.

Typically, ectomycorrhizal pine seedlings have foliage with more than 15 ppm Zn (Boyer and South 1985, Flinn et al. 1980, Jalkanen and Rikala 1995, Knight 1976). When

soil contains less than 1 ppm Zn, nonmycorrhizal seedlings may become Zn deficient (i.e., <9 ppm foliar Zn). Nonmycorrhizal pines, with less than 5 ppm Zn in foliage, might outgrow the deficiency after short roots become mycorrhizal. There are no reports of Zn deficiencies for irrigated loblolly pine seedlings grown in “old ground.”

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Address correspondence to:

David South, Emeritus Professor, College of Forestry, Wildlife and Environment, Auburn University, AL 36849; email: southdb@auburn.edu.

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Table 1. Effect of phosphoric acid (183 kg P/ha) on foliar and soil analysis of loblolly pine seedlings at the Union Camp Nursery

Table 1a. Foliar samples collected on August 18, 1986

Measured variable	N (%)	S (%)	P (%)	K (%)	Mg (%)	Ca (%)	B (ppm)	Zn (ppm)	Mn (ppm)	Cu (ppm)	Fe (ppm)	Na (ppm)	Al (ppm)
Acid	2.34	0.12	0.25	0.97	0.07	0.23	93	8.4	686	6	172	300	370
Control	2.32	0.09	0.08	1.02	0.11	0.28	86	7.2	728	8	232	320	590
P>F	0.629	0.007	0.001	0.085	0.001	0.019	0.77	0.07	0.043	0.57	0.038	0.37	0.001
LSD ₀₅	0.11	0.01	0.04	0.06	0.01	0.04	64	1.3	39	6	54	55	71

Table 1b. Foliar samples collected on November 26, 1986

Measured variable	N (%)	S (%)	P (%)	K (%)	Mg (%)	Ca (%)	B (ppm)	Zn (ppm)	Mn (ppm)	Cu (ppm)	Fe (ppm)	Na (ppm)	Al (ppm)
Acid	1.86	0.088	0.21	0.45	0.126	0.30	38	103	1,140	5	281	240	534
Control	2.14	0.106	0.21	0.52	0.144	0.36	44	93	1,264	4	234	180	506
P>F	0.001	0.313	0.951	0.033	0.037	0.074	0.27	0.58	0.084	0.55	0.085	0.37	0.300
LSD ₀₅	0.22	0.04	0.08	0.06	0.016	0.069	13	48	150	2	58	166	65

Table 1c. Soil samples collected on November 26, 1986

Measured variable	pH	S (%)	P (%)	K (%)	Mg (%)	Ca (%)	B (ppm)	Zn (ppm)	Mn (ppm)	Cu (ppm)	Fe (ppm)	Na (ppm)	OM (%)
Acid	5.1	4	73	20.6	27	98	0.6	5	15	1.7	34	10	1.5
Control	5.2	8	44	25.4	28	112	0.6	2	11	1.2	22	12	1.2
P>F	0.313	0.28	0.007	0.042	0.326	0.18	0.70	0.18	0.003	0.44	0.009	0.24	0.530
LSD ₀₅	0.4	8	16	4.5	9	24	0.3	4	1.8	1.6	4	3	1.2

Foliar samples (from five control plots and five treated plots) were collected on August 18 and November 26, 1986. Soil samples were collected on November 26, 1986, using the Mehlich 3 soil test. The least significant difference (LSD) values are provided at the 0.05 level of probability. Foliar samples collected in August were below 8 ppm Zn from two treated plots and three untreated plots.

P>F = probability of a greater F value

Abbreviations

Al = aluminum
B = boron
Ca = calcium

Fe = iron
Mg = magnesium
Mn = manganese

N = nitrogen
Na = sodium
OM = organic matter

P = phosphorus
S = sulphur
Zn = zinc

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