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Zinc fertilization in bareroot pine seedbeds

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#### Abstract

Zinc deficiencies are rare in pine seedlings with less than five documented cases in bareroot nurseries. One temporary deficiency occurred after soil was land-leveled (i.e., topsoil removed) and another occurred on a peat soil after more than 2,200 kg of agricultural lime was applied before sowing. Farmers also observe zinc deficiencies on (1) over-limed areas and (2) where Zn-demanding crops are grown on areas where topsoil was removed during land leveling. Since ZnSO<sub>4</sub> is a naturally occurring pesticide, sometimes height growth increases are due to pest control. In pathogen-rich soils, pine growth may be improved more by the fungicidal effect than by a growth benefit from added sulphur and zinc. As a result, a pseudo-deficient response is possible when growth of non-deficient seedlings increases after treatment with large amounts of ZnSO<sub>4</sub> or ZnCl<sub>2</sub>. In some trials, claims of a Zn deficiency have been made without supporting evidence from foliar tests or from tests using pathogen-free soil. Although fertilization with Zn increased seedling growth at pine nurseries in New Zealand, India, Russia, and Wisconsin, only at the Sweetwater Nursery in New Zealand did foliar tests prove a Zn deficiency.

#### Keywords

Nutrition; Zinc; Foliar analysis; Soil testing; Land leveling; Mycorrhiza

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### 1 Introduction

Although Zn-deficiencies in endomycorrhizal crops might be the most ubiquitous micronutrient deficiency worldwide (Alloway 2008), deficiencies in bareroot pine nurseries are rare. Since corn (see Table 1 for scientific names) is a Zn-inefficient species, deficiencies may occur at some farms while no deficiency is present on pine seedlings growing in adjacent irrigated nurseries (on the same soil series). When bareroot seedlings are growing in irrigated soil, ectomycorrhizal conifers rarely need to be fertilized with Zn. Although conditions for a Zn deficiency in pine plantations are rare in the Northern Hemisphere, deficient stands have occurred in Australia, New Zealand, and South Africa. In South Australia, zinc deficiency was an unknown problem for over 5 decades (Boardman and McGuire 1990). One theory is that rapid-growth of exotic pines (e.g. 20 m<sup>3</sup> ha<sup>-1</sup> year<sup>-1</sup>) increases the chance of foliar concentrations dropping below 10  $\mu$ g g<sup>-1</sup> Zn. Although several reviews have been published (Stone 1968; Boardman and McGuire 1990; Alloway 2008; Noulas et al. 2018), this paper pertains to Zn use in bareroot pine seedbeds.

### 2 History

Researchers in Germany determined that Zn sulphate (ZnSO<sub>4</sub>) could kill various species without harming conifers (Baumann 1885; Bourcart 1913). This discovery encouraged herbicide trials with Zn rates exceeding 180 kg ha<sup>-1</sup> (Table 2). Likewise, ZnCl<sub>2</sub> (1,525 kg ha<sup>-1</sup>) was tested as a fungicide at ten nurseries with promising results at two nurseries (Hartley and Pierce 1917). In some trials, Zn increase germination of pine seed. Coating unstratified seed with ZnO increased germination of *Pinus resinosa* and *Pinus banksiana* by 11% (Johnson 1946) and a high rate of ZnSO<sub>4</sub> increased germination of *Pinus palustris* at a nursery in Bogalusa, Louisiana (Wakeley 1927). Since nursery managers currently treat pine seed with other fungicides, use of ZnO to control seed fungi has declined. However, Zn is a component of some fungicides like mancozeb, ziram and zineb. In one greenhouse trial (Allen et al. 2004), soaking pine seed with mancozeb (0.9% Zn and 7.4% Mn) before sowing, increased germination of *Pinus palustris* by 18%. At the Ashe Nursery in Mississippi foliar applications of ziram (21% Zn) reduced rust infection on *Pinus elliottii* seedlings (Siggers 1951).

When applied at herbicide rates, ZnCl<sub>2</sub> reduced grass populations at the Halsey Nursery in Nebraska (Hartley and Pierce 1917) while ZnSO<sub>4</sub> reduced weeds at nurseries in Montana (Kitchin 1920; Wahlenberg 1930) and Australia (Adams 1951; Richards 1956). Although high rates of ZnSO<sub>4</sub> reduced germination of slash pine (McKellar 1936; Richards 1956), the reduction in weeding times made the treatment economically attractive. After effective herbicides became available, testing of Zn compounds declined (Richards 1956; Stoeckeler and Jones 1957).

[Abbreviations: AN = ammonium nitrate. B = boron. Ca = calcium. Cl = chloride. Cu = copper. DAP = diammonium phosphate. EDTA = ethylenediaminetetraacetic acid. Fe = iron. K = potassium. MAP = monoammonium phosphate. Mg = magnesium. Mn = manganese. N = nitrogen. Na = sodium. P = phosphorus. S = sulphur. TSP = triple superphosphate. Zn = zinc. Soil pH was measured in water.]

Species	Common name	Mean	Min	Max	Reference
NURSERY		µg g⁻¹	µg g⁻¹	µg g⁻¹	
Pinus contorta Douglas ex Loudon	Lodgepole	258	65	425	Landis 1976b
P. echinata Mill.	Shortleaf	176	66	300	Berry and Marx 1976
P. elliottii Engelm.	Slash	85	60	180	Munson 1982
P. palustris Mill.	Longleaf	38	18	69	Starkey and Enebak 2012
P. pinaster Aiton	Maritime	26	15	36	Mañas et al. 2009
P. ponderosa Lawson & C. Lawson	Ponderosa	60	38	96	Mexal and Fisher 1987
P. ponderosa Lawson & C. Lawson	Ponderosa	38	11	82	Landis 1988
<i>P. radiata</i> D. Don	Monterey	19	15	22	Knight 1975a
<i>P. radiata</i> D. Don	Monterey	51	36	72	Knight 1978
<i>P. radiata</i> D. Don	Monterey	32	19	45	Flinn et al. 1980
<i>P. radiata</i> D. Don	Monterey	18	2	50	Knight 1976
<i>P. radiata</i> D. Don	Monterey	122	105	161	Richardson and Perkins 1985
<i>P. radiata</i> D. Don	Monterey	40	25	57	Hopmans and Flinn 1983
P. resinosa Aiton	Red	95	80	110	lyer and Wilde 1974
P. rigida Mill.	Pitch	46	40	53	Berry 1982
P. strobus L.	Eastern white	97	83	109	lyer et al. 2002
P. sylvestris L.	Scots	73	38	140	Jalkanen and Raitio 1995
P. sylvestris L.	Scots	94	52	171	Raitio 1983
P. taeda L.	Loblolly	43	33	62	Marx 1990
P. taeda L.	Loblolly	61	41	101	Danielson 1966
P. taeda L.	Loblolly	55	30	87	Boyer and South 1985
P. taeda L.	Loblolly	55	31	115	Starkey and Enebak 2012
P. taeda L.	Loblolly	64	52	74	South et al. 2018
P. taeda L.	Loblolly	42	38	45	South et al. 2017
P. taeda L.	Loblolly	47	4	60	South 2024
P. taeda L.	Loblolly	111	47	163	Berry 1985
P. thunbergi Parl.	Japanese black	80	66	93	Hathaway and Whitcomb 1984
GREENHOUSE					
Zea mays L.	Corn	13	11	15	Banik et al. 2021
P. banksiana Lamb.	Jack	61	53	73	MacDonald et al. 1986
P. elliottii Engelm.	Slash	35	16	61	Van Lear and Smith 1972
P. elliottii Engelm.	Slash	49	46	52	McKee 1976
P. elliottii Engelm.	Slash	39	22	71	Buchler 2002
<i>P. jeffreyi</i> Balf.	Jeffery	170	95	243	Walker and Kane 1997
P. patula Schiede ex Schltdl. & Cham.	Mexican weeping	38	26	44	Buchler 2002
P. sylvestris L.	Scots	55	18	112	Goslin 1959
P. taeda L.	Loblolly	21	12	28	Buchler 2002
P. taeda L.	Loblolly	11	4	29	Zillmer 1978

Although ZnSO<sub>4</sub> stimulated growth of some plants (Bourcart 1913), the need for Zn as a plant nutrient was questioned until about 1926 (Chandler 1937; Sommer and Lipman 1926). Growth gains from ZnSO<sub>4</sub> might be due to overcoming a S-deficiency or from suppressing seed-borne pathogens, rather than remedying a Zn-deficiency. In Australia, foresters applied ZnCl<sub>2</sub> to correct a deficiency in pine (Kessell and Stoate 1936) which supported claims that Zn is an essential element. Due to positive results, ZnSO<sub>4</sub> was tested on pines at the Vallonia Nursery in Indiana in 1937 (Auten 1945) and at a greenhouse in 1948 (Voigt et al. 1958). At nurseries in the United Kingdom, no benefit was observed from applying ZnSO<sub>4</sub> to seedbeds (Benzian 1965). The first operational use of a micronutrient-blend containing Zn may have been at the Ashe Nursery in Mississippi in 1964. Although there was no evidence of a Zn deficiency (Maki and Henry 1951), a slow-release treatment (FRIT 503) was applied at a rate of 33.6 kg ha<sup>-1</sup> (1.9 kg Zn ha<sup>-1</sup>). In North America, most Zn trials in bareroot pine nurseries ceased by 1970. Zn deficiencies at nurseries occurred in New Zealand (Knight 1976) and Alabama (South 2024), and possible deficiencies occurred in Wisconsin (Tanaka et al. 1967) and Russia (Agnistikova and Scerbakov 1960).

Table 2. Pine response to zinc (Zn) treatments in trials involving herbicides (H), fungicides (F), or micronutrients (M). See Table 1 for species' scientific names. \* = significantly different  $\alpha = 0.07$ .

Zinc		Common name	Variable	% response	Reference
BAREROOT					
731 kg ha <sup>-1</sup>	F	Jack	Number	+352	Hartley and Pierce 1917
67 kg ha <sup>-1</sup>	М	Shortleaf	Dry mass	+ 101	Auten 1945
2.3 kg ha <sup>-1</sup>	М	Scots	Dry mass	+16	Agnistikova and Scerbakov 1960
300 kg ha <sup>-1</sup>	Н	Longleaf	Number	+11	Wakeley 1927
226 kg ha <sup>-1</sup>	Н	Red	Height	+10	Hyland 1929
3,500 mg/L	М	Monterey	Height	+9	Knight 1976
300 kg ha <sup>-1</sup>	Н	Western white	Number	+8	Wahlenberg 1930
162 kg ha <sup>-1</sup>	F	Monterey	Number	+1	Ram Reddy and Misra 1970
316 kg ha <sup>-1</sup>	Н	Monterey	Height	-10	Bibby 1953
182 kg ha <sup>-1</sup>	F	Eastern white	Number	-14	Hansen et al. 1923
194 kg ha <sup>-1</sup>	Н	Slash	Height	-20	McKeller 1936
206 kg ha <sup>-1</sup>	Н	Slash	Number	-28*	Richards 1956
GREENHOUSE					
1 mg/pot	М	Mexican weeping	Height	+18*	Bari and Gupta 1970
13.6 mg/pot	М	Slash	Dry mass	+15	Van Lear and Smith 1972
160 mg/pot	М	Mexican weeping	Dry mass	+14	Buchler 2002
6.8 mg/pot	М	Slash	Dry mass	+7*	McKee 1976
226 kg ha <sup>-1</sup>	Н	Red	Height	0	Hyland 1929
7.8 kg ha <sup>-1</sup>	М	Loblolly	Dry mass	0	Richards 1961
3.9 kg ha <sup>-1</sup>	М	Red	Color	0	Voigt et al. 1958
10 mg/pot	М	Lobiolly	Diameter	-9	Zillmer 1978
40.8 mg/pot	М	Slash	Dry mass	-7	Van Lear and Smith 1972
160 mg/pot	М	Mexican weeping	Dry mass	-26	Buchler 2002
FIELD					
4,800 mg/L	М	Monterey	Height	+118*	Kessel and Stoate 1936
5.6 g/tree	М	Monterey	Height	+21*	Thorn and Robertson 1987
1 g/tree	М	Mexican weeping	Height	+15	Vail et al. 1961
8.4 kg ha⁻¹	М	Loblolly	Dry mass	+4	Sypert 2006
40 g/tree	М	Monterey	Color	0	Weston 1956
4.6 kg ha⁻¹	М	Scots	Height	0	Veijalainen 1983
22.5 g/tree	М	Monterey	Height	0	McGrath 1978
28 kg ha <sup>-1</sup>	М	Slash	Volume	0	Jokela et al. 1991
3.3 kg ha⁻¹	М	Slash	Volume	0	Vogel and Jokela 2011
1 g/tree	М	Monterey	Height	-4	Vail et al. 1961
7 g/tree	М	Monterey	Height	- 4	Ruiter 1983
8.4 kg ha <sup>-1</sup>	М	Loblolly	Dry mass	-9	Sypert 2006
3.3 kg ha <sup>-1</sup>	М	Loblolly	Volume	-11	Vogel and Jokela 2011
50 kg ha⁻¹	М	Monterey	Height	-26	Lange 1969

Although soil levels of zinc could be measured (Tanaka et al. 1967), most nursery soil tests before 1983 did not include zinc. Soil tests for Zn and other micronutrients were considered too costly for routine analyses of nursery soils (lyer and Love 1974). Generally, Zn deficiencies either did not occur (Maki and Henry 1951; Anderson 1968; Youngberg 1984; Maxwell 1988) or were overlooked at nurseries where they did occur. At established nurseries, rarely do bareroot pine seedlings have foliage with less than 10  $\mu$ g g<sup>-1</sup> (Table 1). In Colorado, some seedlings with high foliar Ca had less than 11  $\mu$ g g<sup>-1</sup> Zn in foliage (Landis 1988) and on leveled new ground in Alabama, some pine seedlings had foliage with less than 6  $\mu$ g g<sup>-1</sup> Zn (South 2024).

At a nursery in Russia, applying Zn (10 kg ha<sup>-1</sup> ZnSO<sub>4</sub> •7H<sub>2</sub>O) before sowing increased growth of pine seedlings (Agnistikova and Scerbakov 1960) and in Wisconsin, a foliar spray ( $10 \ \mu g g^{-1} ZnSO_4 \cdot H_2O$ ) stimulated foliar growth of pine (Tanaka et al. 1967). However, the reason for increased growth is not known as levels of Zn and S in the foliage were not reported. In New Zealand, a foliar spray of ZnSO<sub>4</sub> corrected visible Zn deficiency symptoms and increased foliar Zn concentrations (Knight 1976).

### 3 Soil tests

Total soil Zn is the sum of organically-bound, minerally-bound and extractable Zn (Chowdhury et al. 1977). In the southern United States Coastal Plain, total Zn in the top 10 cm of soil is typically less than 18  $\mu$ g g<sup>-1</sup> (Figure 1). When growing pine seedlings, the concern is not for the total amount, but for the amount that is available to plants. For example, a peat soil with 88% organic matter had 4  $\mu$ g g<sup>-1</sup> total Zn, but after lime was applied, the nursery produced Zn-deficient seedlings (Knight 1976). This illustrates why laboratories report an estimate of Zn available to plants.

In *Pinus taeda* plantations, volume growth was not correlated with soil Zn (Mehlich 3; r = 0.05; NCSFNC 1992). Even so, at one nursery, seedling height was correlated with soil Zn (r = 0.84; South et al. 2018b). It seems likely this correlation was driven by overall soil fertility. Occasionally, there is a positive correlation between extractable K and Zn. In pine plantations, this correlation was r = 0.53 (NCSFNC 1992) and, at one nursery in Texas, the correlation was r = 0.87 (South et al. 2018b). At other locations, K-Zn correlations in soil are not significant.

In fertilized and irrigated nursery soils, extractable Zn may range from 0.6 to 8  $\mu$ g g<sup>-1</sup> but in unfertilized soils, the amount can be as low as 0.1 or 0.2  $\mu$ g g<sup>-1</sup> (Tanaka et al. 1967; NCSFNC 1992). In some regions with sufficient soil Zn, laboratories might not analyze samples for Zn. When soil tests are available, most agronomists do not recommend Zn fertilization unless soil levels drop below 1 or 2  $\mu$ g g<sup>-1</sup> (Figure 2). In Australia, Zn deficiencies occur on deep sands (Turner and Lambert 1986). In a Zn-deficient sand, only 0.2  $\mu$ g g<sup>-1</sup> Zn is required for good growth of eucalyptus (Wallace et al. 1986).



Figure 1. Soils in the Pacific Northwest usually have more total zinc than soils of the Coastal Plain of the southeastern United States (Smith et al. 2019). Credit: U.S. Geological Survey Department of the Interior/USGS. A map for Europe is available at: <u>https://ars.els-cdn.com/content/image/1-s2.0-S0946672X17308386-gr1.jpg</u>.



Figure 2. Various opinions exist regarding how much Zn fertilization to apply to pine seedbeds. When a Mehlich 3 test indicates soil contains 0.5  $\mu$ g g<sup>-1</sup> S, one agronomist (orange line) might recommend 2.5 kg ha<sup>-1</sup> of Zn, another might apply 3.4 kg ha<sup>-1</sup> (green line) and a third might suggest applying 4.5 kg ha<sup>-1</sup> (blue line).

### 4 Tissue analysis

Soil tests are used to determine fertilization rates before sowing while a foliar analysis can verify a suspected Zn deficiency. Managers may use a  $10-\mu g g^{-1}$  threshold to determine if a foliar application of Zn should be applied to pine seedlings. Without data, others have proposed a  $30 \ \mu g g^{-1}$  Zn threshold, but this high value would likely waste both time and money (Simpson and Grant 1991). Due to a lack of research, the

Zn threshold for *Pinus taeda* pine foliage is not known but a proposed "adequate range" (i.e. no stunting) is 10 to 300  $\mu$ g g<sup>-1</sup> Zn. Although a "survey range" for *Pinus taeda* goes from 23 to 160  $\mu$ g g<sup>-1</sup> Zn (Figure 3), the "adequate range" is 10 to 300  $\mu$ g g<sup>-1</sup>. In plantations in Australia, deficient pines had needles with 5 to 9  $\mu$ g g<sup>-1</sup> Zn while healthy trees had 10 to 19  $\mu$ g g<sup>-1</sup> (Kessell 1943). As a result, a 10- $\mu$ g g<sup>-1</sup> Zn threshold was adopted for the beginning of the "adequate range".

Although some believe adequate Zn concentrations are lower for bareroot seedlings than for container-grown pine seedlings (Weetman and Wells 1990; Timmer 1991; Landis 1998; Hawkins 2011), data do not support this belief (Hathaway and Whitcomb 1984; Starkey and Enebak 2012). The proposed 30 to 150  $\mu$ g g<sup>-1</sup> range for Zn in container-grown stock was obtained from a fertilizer company that produced slowrelease fertilizers (Landis 1985) while a "survey range" of 10 to 125  $\mu$ g g<sup>-1</sup>Zn for bareroot seedlings was derived, in part, from needles sampled from unfertilized trees in the forest (Powers 1974). There is no biological reason why slow-release fertilizers (Walker and Kane 1997) or foliar sprays (Grunes et al. 1961) would increase the 10  $\mu g g^{-1} Zn$ threshold for pine seedlings grown in pots. Research results using containers in greenhouses (Buchler 2002; Van Lear and Smith 1976) do not support the idea that 16 to 22  $\mu$ g g<sup>-1</sup> Zn in foliage is inadequate for container-grown slash pine. Based on data from publications listed in Table 1, the revised tentative range for "adequate" foliar Zn is 10 to 300  $\mu$ g g<sup>-1</sup> for both bareroot and container-grown stock. The "sufficiency" range for certain vegetables is 20 to 250  $\mu$ g g<sup>-1</sup> Zn (Noulas et al. 2018). Some might argue that foliage with 5 to 9  $\mu$ g g<sup>-1</sup> Zn is within the "adequate" range (Knight 1976; Ruiter 1983). In Australia, some Cu-deficient pines had 8  $\mu$ g g<sup>-1</sup>Zn in foliage (Ruiter 1969) while pines in South Africa had 3 to 6  $\mu$ g g<sup>-1</sup> Zn in foliage (Grey 1988).



Figure 3. Needles collected from nursery-grown *Pinus taeda* seedlings in December-January may range from 23 to 160  $\mu$ g g<sup>-1</sup> Zn. The median values for blue and orange bars from different collection years are 55 and 56  $\mu$ g g<sup>-1</sup> Zn, respectively (Boyer and South 1985; Starkey and Enebak 2012).

#### 4.1 Deficiency symptoms

Zn deficiency symptoms in pine seedlings include: stunting, short needles, rosette buds, dark-green or bronze needles. When grown in water, deficient *Pinus taeda* seedlings appear stunted with short needles (Figure 4) while *Pinus radiata* seedlings are stunted with short, dark-green needles (Smith and Bayliss 1942). In soil, *Pinus radiata* seedlings develop a rosette of buds in place of the usual single bud and foliage may

exhibit a bronze color (Figure 5). Zn is transported slowly in pines (McGrath and Robson 1984) so stunting and rosette buds are two symptoms. Photos of Zn-deficient seedlings have been published for pine (Will 1985) and spruce (van den Driessche 1989). Several handbooks provide images of Zn-deficient row-crops (Alloway 2008; Bryson and Mills 2014; Barker and Eaton 2015).



Figure 4. *Pinus taeda* seedlings grown in water culture in a greenhouse. Deficient seedlings had short, thick, and twisted needles with a color of 7.5 GY 4/4, 4/6 that is darker than the darkest color considered normal (Lyle 1969).



Figure 5. *Pinus radiata* seedlings from the Sweetwater Nursery in New Zealand (Will 1985). Zn-deficient seedlings are on the right while those on the left were likely treated with a 10,000 µg g<sup>-1</sup> solution of ZnSO<sub>4</sub>•7H<sub>2</sub>O. Photo provided by Scion New Zealand (Photo by H. Hemming).

#### 4.2 Threshold level in foliage

A "critical level" for a nutrient is defined as the foliar concentration that occurs at 90% of maximum yield (Ulrich 1948; Ulrich and Hills 1967; Bates 1971). In Europe, the equivalent term is "threshold" (Stefan et al. 1997). Although threshold values of 10 to 12  $\mu$ g g<sup>-1</sup> Zn were developed using *Pinus radiata* seedlings with dry mass > 3 g (Raupach 1975; McGrath and Robson 1984b), critical levels for southern pines are not known (Allen 1987; Weetman and Wells 1990; NCSFNC 1992; Boardman et al. 1997). Zn threshold values developed from seedlings that weigh less than 0.2 g are not useful for evaluating the nutrient needs of 9-month-old seedlings (McGrath and Robson 1984).

In a bareroot nursery, pine foliage with 9  $\mu$ g g<sup>-1</sup> appeared normal (Figure 6) while deficient seedlings had 4  $\mu$ g g<sup>-1</sup> (Knight 1976). Brockley (2001) suggested *Pinus contorta* foliage with 9  $\mu$ g g<sup>-1</sup> Zn was probably deficient while others believed 20  $\mu$ g g<sup>-1</sup> Zn is the threshold value for *Pinus sylvestris* (Talkner et al. 2019). Without data from a response curve, "critical value" estimates are dubious, especially when authors obtain values derived from a distribution curve generated from foliar sampling.



Figure 6. Foliar nitrogen (N) and zinc (Zn) concentrations from bareroot *Pinus taeda* and *Pinus radiata* (Sweetwater) nurseries (Knight 1976; Starkey and Enebak 2012). Due to carbohydrate dilution, N and Zn concentrations were lower in October than in July, but Zn concentrations remained relatively constant from October to February (Starkey and Enebak 2012). The median concentration for foliar Zn in July, October and February were 77, 56 and 56 μg g<sup>-1</sup> Zn, respectively. Dashed black line represents a 9 μg g<sup>-1</sup> threshold for Zn deficiency.

#### 4.3 Pseudo-deficiency

A pseudo-Zn-deficiency occurs when foliar Zn levels for pine seedlings are adequate (> 9  $\mu$ g g<sup>-1</sup> Zn) but growth is improved after Zn treatments reduce populations of weeds (Bourcart 1913), nematodes (Korthals et al. 2000), or fungi (El-Fawy and El-Said 2018; Bastakoti 2023). Possible examples of a pseudo-Zn-deficiency include high Zn rates applied in bareroot nurseries (Table 2) plus two greenhouse trials (Anderson 1967; McKee 1976). Although foliar and pathogen analyses were not conducted (Anderson 1967), some say root growth increased "presumably because of the destruction of soilborne pathogens" (Duffield and Eide 1962). Likewise, since ZnSO<sub>4</sub> can reduce mycelial growth and increase height of *Sesamum indicum* L. (EI-Fawy and EI-Said 2018), a fungicidal effect might also explain a growth response at the Boscobel Nursery (Tanaka et al. 1967). Due to growth benefits from controlling various nursery pests, foliar analyses of both Zn and S should be a requirement before making a Zn-deficiency claim.

### 5 Soils

Prior to sowing seed, many bareroot nurseries in the southern United States have soils that contain less than 3  $\mu$ g g<sup>-1</sup> of extractable Zn (Figure 7) and some seedbeds in Wisconsin have less than 1  $\mu$ g g<sup>-1</sup> Zn (Tanaka et al. 1967). Due to low levels and variability, there is no correlation between soil Zn (Mehlich 1) and sand content in nursery soils (South and Davey 1983). Since Zn does not readily leach, the topsoil often has more Zn than subsoil (Thorne 1957).





#### 5.1 Soil pH

In theory, Zn availability to plants increases as soil acidity increases. In a greenhouse using pots filled with a Bladen series soil (clayey, mixed, thermic Typic Ochraqualt), soil adjusted to pH 4.5 and pH 7.8 had extractable Zn levels (Mehlich 1) of 2.0 and 0.7  $\mu$ g g<sup>-1</sup>, respectively (Yawney et al. 1982). In contrast, at several bareroot nurseries, available Zn in acid soils was positively correlated with soil pH (Figure 8). The slopes for the correlations in sandy nurseries vary but sometimes the slope can be +0.7  $\mu$ g g<sup>-1</sup> Zn per unit of pH increase.





An increase in foliar Zn concentration is to be expected when lime reduces seedling growth without affecting Zn uptake. When Zn in pine needles drops below 12  $\mu$ g g<sup>-1</sup>, soil may be above pH 7.0 (Ruiter 1983; Landis 1988) or below pH 6.0 (McGrath 1978; South et al. 1988).

In one trial (pH 4.8 soil), applying lime reduced growth of pine (soil pH 6.8) and increased foliar Zn levels by 17  $\mu$ g g<sup>-1</sup> (Marx 1990). When growing in sand (75% organic matter V/V), adding dolomitic lime reduced pine growth, and increased foliar Zn by 21  $\mu$ g g<sup>-1</sup> (Hathaway and Whitcomb 1984). When pine roots were growing in water instead of soil, pH of the solution was modified with KOH and pH had no effect on foliar Zn concentration (Zhang et al. 2015).

A Zn deficiency occurred at a peat nursery (88% organic matter and 12% mineral soil) at pH 4.2 (Knight 1975a). It is likely this deficiency (Figure 9) resulted from over liming a high-organic matter soil (Knight 1975b, Will 1985). For example, when soil organic matter was less than 1% at pH 3.9, liming did not induce a Zn deficiency at a nursery in Texas (South et al. 2017).

Due to poor seedling growth, Fe deficiency and inconsistent ectomycorrhiza, several pine nurseries on alkaline soils have closed. At the Albuquerque Nursery in New Mexico (pH 7.2 to 8.2), pine foliage had 19 to 96  $\mu$ g g<sup>-1</sup> Zn (Mexal and Fisher 1987; Landis 1988). On a calcareous soil in Greece, pines were also not Zn deficient and foliage contained 25 to 51  $\mu$ g g<sup>-1</sup> Zn (Michopoulos et al. 2017). Although several agronomic crops growing on calcareous soils become Zn-deficient when soil pH exceeds 7.0, pine seedlings usually obtain adequate Zn on alkaline soils.



Figure 9. Soil at the Sweetwater Nursery contained 88% peat and 12% mineral soil. Soil ranged from pH 4.0 to 4.7 and peat contained 5,200 to 10,100  $\mu$ g g<sup>-1</sup> Ca and 2 to 5  $\mu$ g g<sup>-1</sup> Zn. Deficient *Pinus radiata* pine seedlings with bronze-colored needles had 2 to 5  $\mu$ g g<sup>-1</sup> Zn in foliage while green-colored foliage had 9 to 20  $\mu$ g g<sup>-1</sup> Zn. Photo provided by Scion New Zealand. (Photo P.J. Knight).

#### 5.2 Land leveling

When preparing land for a new pine nursery, topsoil is often removed and stockpiled before land leveling operations begin. When leveling is completed, topsoil is replaced but the stockpiled soil may not be sufficient to cover all areas. Land leveling can remove mycorrhizal spores, produce P-deficiencies (Trappe and Strand 1969) and can lower available Zn to less than 1.2  $\mu$ g g<sup>-1</sup> (Grunes et al. 1961). Land leveling contributes to Zn deficiency in corn (Grunes et al. 1961; Shapiro 2008) and caused P and Zn deficiencies on pine at the Union Camp Nursery in Alabama. Land was leveled in July 1985 and soil was fumigated in March 1986 (South et al. 1988). Seedlings were mostly P-deficient but in some areas, seedlings were also Zn deficient. Since P-deficiency symptoms include stunting and purple needles, seedlings stunted due to a Zn-deficiency were overlooked (South 2024). Even without soil fumigation, land leveling can result in stunted, endomycorrhizal crops (Grunes et al. 1961).

#### 5.3 Organic matter

Sometimes organic matter is added before or after sowing in hopes of replacing some micronutrients removed by harvesting seedlings. Applying a mulch after sowing

can add 0.1 to 0.6 kg ha<sup>-1</sup> of Zn (Mexal and Fisher 1987; dos Santos 2006; Kilmek et al. 2012). However, little of this Zn is readily available to seedlings.

Although operational nurseries avoid applying sludge to seedbeds, researchers may test the effects of sewage sludge on growth of bareroot pines. Typically, the addition of sludge increases pine growth and foliar Zn concentrations (Berry and Marx 1976; Mexal and Fisher 1987; Selivanovskaya and Latypova 2006). For example, 275 tonnes ha<sup>-1</sup> of sludge doubled stem diameter of *Pinus echinata* and increased foliar Zn to 300  $\mu$ g g<sup>-1</sup> Zn. In plywood microplots, adding 6.8 kg m<sup>-2</sup> of sludge produced foliage that ranged from 80 to 143  $\mu$ g g<sup>-1</sup> Zn (Berry 1985). Results from the microplots indicate two sludge sources decreased heights, two increased heights and one produced no significant effect. Results from various studies indicate foliar Zn levels ranging from 150 to 350  $\mu$ g g<sup>-1</sup> are not harmful to growth of pine seedlings. In soils without sludge, foliage concentrations rarely exceed 150  $\mu$ g g<sup>-1</sup> Zn unless foliage contains residue from Zn sprays. Risk-adverse managers chose to not risk losing profits by adding sewage sludge before sowing pine seed.

#### 5.4 Phosphorus

Although elevated soil phosphorus levels can hinder the absorption of Zn in endomycorrhizal crops (Thorne 1957; Marschner 1993; Barker and Eaton 2015), Zn deficiencies might not exist after application of phosphorus fertilizers in pine nurseries. In contrast, an Fe deficiency can occur after an excessive applications of  $Ca(H_2PO_4)_2$ (Stienbeck et al. 1966). Instances of chlorotic needles and stunted pine seedlings can arise when foliage becomes Fe-deficient due to excessive Ca (Landis 1988; Zhang et al. 2015; South 2022). In some trials, it is not entirely clear if Fe chlorosis is caused primarily by added P or added Ca. When a Zn deficiency occurred at a pine nursery, it was likely due to applying too much lime (Will 1985).

The application of Ca and P to nursery soil (2,976 kg ha<sup>-1</sup> of TSP) induced Cu and Zn deficiencies in hybrid poplar (Teng and Timmer 1990). However, in bareroot nurseries, lower rates of TSP (110 to 165 kg ha<sup>-1</sup>) are used when soil contains less than 45 µg g<sup>-1</sup> P (Mehlich 3), (Davey and McNabb 2019). For bareroot pine nurseries, TSP is not routinely applied to the soil, possibly because of adequate P levels in soil and foliage (Donald 1991). When a lack of mycorrhiza is detected, some managers spray liquid P fertilizers to enhance seedling growth (South et al. 2018a). While TSP was previously favored by nursery managers (South and Zwolinski 1996), liquid P fertilizers are now preferred by managers.

It should be noted that there are instances where P fertilization did not inhibit the uptake of Zn. For example, in potted pine exposed to rain, fertilization with TSP (1,000  $\mu$ g g<sup>-1</sup> P) increased foliar Zn by 35  $\mu$ g g<sup>-1</sup> (Bays 2022). Additionally, in several greenhouse trials, P fertilization did not decrease foliar Zn concentrations in pine (Van Lear and Smith 1972; Smilde 1973; Hook et al. 1983; Saur 1989). Similarly, a test with phosphoric acid (South et al. 1988) showed that P fertilization did not reduce Zn uptake. Some P fertilizers also contain Zn which can increase foliar Zn. TSP may contain 61  $\mu$ g g<sup>-1</sup> Zn (Raven and Loeppert 1997) and MAP from North Carolina may contain 870  $\mu$ g g<sup>-1</sup> Zn (Lambert at al. 2007).

There are some cases where a combination of Ca and P can reduce Zn uptake. In a trial with 3-year-old pines, fertilization with 267 kg ha<sup>-1</sup> of TSP (13.6% Ca and 20% P) led to a 14- $\mu$ g g<sup>-1</sup> reduction in foliar Zn concentration (Saur 1989). Nevertheless, even after this reduction, the foliage still contained 44  $\mu$ g g<sup>-1</sup> Zn, which is sufficient for pine growth. Nursery managers may induce a Zn deficiency by applying 2,000 kg ha<sup>-1</sup> of TSP, but they need not worry about causing a Zn deficiency when applying dilute phosphoric (H<sub>3</sub>PO<sub>4</sub>) or phosphorus (H<sub>3</sub>PO<sub>3</sub>) acid to promote seedling growth (Auten 1945; South et al. 1988; Teng and Timmer 1995; Rolando et al. 2014; Woodruff et al. 2014).

For healthy *Pinus taeda* seedlings, P/Zn ratios in foliage ranged from 23 to 65 in the summer and 13 to 48 in the winter (Starkey and Enebak 2012). Opinions about the "expected" ratio for pine vary from 20 to 71 but the 71 ratio is outside the normal range for pine. Most foliar Zn averages are above 30  $\mu$ g g<sup>-1</sup> (Table 1). Pine seedlings with P/Zn ratios over 60 were chlorotic, but yellow needles were likely due to a Fe-deficiency (Landis 1988). At the Sweetwater Nursery in New Zealand (Knight 1975b), green foliage from "healthy" pine seedlings had a P/Zn ratio of 200 with 19  $\mu$ g g<sup>-1</sup> Zn. For pine seedlings, foliar Zn concentrations alone are more operationally meaningful than P/Zn ratios. For bareroot pine seedlings, Zn deficiencies are not determined by calculating P/Zn ratios.

#### 6 Irrigation water

Deficiencies can occur when irrigation water contains no Zn (Finch and Kinnison 1933; Smith and Bayliss 1942). Water from deep wells typically contains less than 0.06  $\mu$ g g<sup>-1</sup> Zn. About 66% of private wells in North Carolina contain water with less than 0.075  $\mu$ g g<sup>-1</sup> Zn (Eaves et al. 2022). When water contains 0.05  $\mu$ g g<sup>-1</sup> Zn, then 1,000 mm of irrigation will add 0.5 kg ha<sup>-1</sup> of Zn. Although data are not available, a lack of irrigation likely contributed to the Zn deficiency at the Sweetwater Nursery in 1973.

Zinc levels in surface water are variable. Runoff from containment basins at nurseries range from 0.001 to 0.065  $\mu$ g g<sup>-1</sup> Zn (Copes et al. 2017). Rainfall may contain more than 0.01  $\mu$ g g<sup>-1</sup> Zn (Wagner and Holloway 1974; Jeffries and Snyder 1981) and river water might range from 0.01 to 1.2  $\mu$ g g<sup>-1</sup> (Hem 1972).

When seedlings have less than 15  $\mu$ g g<sup>-1</sup> Zn in foliage, managers with no Zn in irrigation water can implement a straightforward test to identify a possible Zn-deficiency. The procedure involves preparing solutions of Zn-sulphate and Cu-sulphate (Lyle 1969). In the late afternoon, any off-color seedlings in a designated plot are treated with Zn-sulfate, while another off-color area is treated with Cu-sulfate. If both treated areas regain normal coloration within two weeks, seedlings were likely deficient in S. A Zn-deficiency is likely if only the ZnSO<sub>4</sub> plot regains normal color. This approach was used at the Boscobel Nursery in Wisconsin (Tanaka et al. 1967).

### 7 Mycorrhiza

Under conditions of adequate available Zn, nonmycorrhizal pine roots can take up enough Zn so seedlings do not become deficient (Colpaert and van Assche 1992; Cumming 1993; Schier and McQuattie 1995; Hartley-Whitaker et al. 2000; Fomina et al. 2006). For example, in July, P-deficient, nonmycorrhizal *Pinus taeda* had 52  $\mu$ g g<sup>-1</sup> Zn in foliage (South et al. 2018a). This suggest that when soil solutions contain sufficient Zn, nonmycorrhizal seedlings can obtain sufficient Zn but they have difficulty obtaining adequate P.

Ectomycorrhizal roots can enhance Zn uptake (Bryson 1980; Sharpe and Marx 1986) and associated mycelia can increase uptake of Zn when soil Zn is insufficient for nonmycorrhizal roots. For example, applying ectomycorrhizal spores to a potting mix

increased foliar Zn levels in container-grown seedlings by 30 to 50  $\mu$ g g<sup>-1</sup> (Walker and Kane 1997).

Soil fumigation can delay ectomycorrhizal formation (Danielson 1966; Munson 1982; South et al. 1988) but when this happens, non-mycorrhizal pine seedlings (700  $\mu$ g g<sup>-1</sup> P in foliage) have purple cotyledons while Zn in foliage is not affected. Typically, soil fumigation has no effect on foliar Zn concentration for ectomycorrhizal pine (Danielson 1966). Fumigation has little or no significant effect on extractable Zn in the soil (Danielson 1966; Ellis et al. 1995; Fraedrich and Dwinell 2003). At one nursery, however, fumigation increased growth of *Pinus elliottii* and, as a result, carbohydrate dilution reduced the concentration of Zn in foliage (Munson 1982). Fumigation with methyl bromide and chloropicrin reduces endomycorrhiza and sometimes this results in Zn-deficiency in broadleaf crops (Wilhelm et al. 1967; LaRue et al. 1975).

### 8 Zn removed at harvest

The amount of nutrients removed by a crop of seedlings depends on the overall mass of seedlings harvested. When seedlings contain 40  $\mu$ g g<sup>-1</sup> Zn, then harvesting 10 Mg of seedlings (dry mass) would harvest 0.4 kg of Zn. For example, harvesting bareroot pine seedlings may remove 0.09 to 0.5 kg ha<sup>-1</sup> of Zn (Knight 1978; Hopmans and Flinn 1983; Boyer and South 1985; Pritchett and Fisher 1987). After harvesting 31 crops of *Pinus taeda*, seedbeds at Courtland, Virginia had Zn levels (Mehlich 3) above 2  $\mu$ g g<sup>-1</sup> Zn (South et al. 2018a). Due to soil dynamics, irrigation, atmospheric deposition, and impurities in lime and fertilizers (Dillard et al. 1982; Raven and Loeppert 1997; Li et al. 2008; Fan et al. 2012; Przybysz et al. 2014; Mikos-Szymańska et al. 2019) most nursery managers need not worry about depleting Zn levels. At the Westvaco Nursery, there was no decline in extractable soil Zn after harvesting four crops of seedlings (Figure 10). In contrast, some speculate that nutrients levels in nursery soils in Wisconsin were low enough to produce a positive response from a foliar treatment of ZnSO<sub>4</sub>.



Figure 10. Soil zinc levels (ammonium acetate extraction) at a bareroot nursery in South Carolina. Field H-1 (>80% sand) was managed with cover-crops from 1983 to early 1989. *Pinus taeda* seed were sown in April of 1989, 1990, 1993, 1994. Soil pH values adjacent to dots represent soil acidity in October-November. Zinc fertilizers were not applied during this period but dolomite was applied in the spring of 1983, 1984, 1988, 1989. 1991 and 1992.

### 9 Toxicity

Pines are relatively tolerant of a solution containing  $10,000 \ \mu g \ g^{-1}$  of  $ZnSO_4$  (Bourcart 1913). As a result, weed control in conifer nurseries with  $ZnSO_4$  was possible because of the "especially high resistance of conifers" to Zn (Wahlenberg 1930). When Zn is applied at high rates, root growth is inhibited before shoot growth (Figures 11 and 12). Zinc toxicity occurred at one conifer nursery when galvanized wire remained on seedbeds for several years (Benzian 1965). Due to concern over the buildup of Zn in soil, managers ceased applying Zn for weed control.

Bareroot pine seedlings treated with sewage sludge will occasionally have 180 to 300  $\mu$ g g<sup>-1</sup> Zn in foliage (Berry and Marx 1976; Munson 1982). Due to Zn tolerance, a tentative "adequate" range for pine needles is 10 to 300  $\mu$ g g<sup>-1</sup> (Berry and Marx 1976; Knight 1976). At a nursery in Colorado, "ideal" *Pinus contorta* seedlings had 265  $\mu$ g g<sup>-1</sup> Zn in foliage (Landis 1976a). Although pine seedlings may tolerate 350  $\mu$ g g<sup>-1</sup> Zn, foliar levels above 500  $\mu$ g g<sup>-1</sup> can reduce growth. In one greenhouse trial, a 50% reduction in pine growth occurred when foliage contained 640 to 1,800  $\mu$ g g<sup>-1</sup> Zn (Beyer et al. 2013). When roots are growing in sandy soil, pine seedlings typically have foliage with less than 200  $\mu$ g g<sup>-1</sup> Zn (Table 1).

Pine seedlings did not show shoot toxicity symptoms when foliage contained less than 320  $\mu$ g g<sup>-1</sup> Zn (Berry and Marx 1976; Landis 1976b). Root initiation and development were numerically greater when container-grown pines had 314  $\mu$ g g<sup>-1</sup> Zn in foliage compared to 116  $\mu$ g g<sup>-1</sup> Zn for untreated seedlings (Mitchell and Fretz 1977). Toxicity symptoms from Zn and Mn occurred when pine foliage contained over 1,000  $\mu$ g g<sup>-1</sup> Zn (Figure 12, Mitchell and Fretz 1977).



Zinc sulphate • 7H<sub>2</sub>O (kg/ha)

Figure 11. In a greenhouse, taproot length was inhibited when ZnSO<sub>4</sub> was applied to sand immediately after sowing pine seed (Hyland 1929). Zinc chloride was more harmful to pine than ZnSO<sub>4</sub> (data not shown).

Root mass 310 mg



Figure 12. A smelter-contaminated soil (930  $\mu$ g g<sup>-1</sup>Zn Mehlich 3 – photo not shown due to dead pine seedlings) and a reference soil (left photo - 11  $\mu$ g g<sup>-1</sup>Zn) were mixed to produced soils with different levels of metals (Beyer et al. 2013). Growth of *Pinus strobus* was not reduced by a soil with 91  $\mu$ g g<sup>-1</sup>Zn (center photo) but a 33% reduction in green mass resulted when soil contained 200  $\mu$ g g<sup>-1</sup>Zn (right photo). Seedlings growing in soil with 200  $\mu$ g g<sup>-1</sup>Zn were likely chlorotic due to a combination of high Zn and manganese (Mn). Photos by Nelson Beyer, United States Geological Survey 2009.

Root mass 270 mg

### **10 Fertilizers**

Several types of Zn compounds are available (Table 3) and common types include ZnO (80% Zn), ZnSO<sub>4</sub> monohydrate (36% Zn), and ZnSO<sub>4</sub> heptahydrate (23% Zn). Since fertilizers contain various concentrations of Zn, adding 10 kg of ZnSO<sub>4</sub> to soil might add 1 kg to 3.6 kg of Zn. Too often researchers report the amount of ZnSO<sub>4</sub> powder applied but fail to mention the amount of Zn applied.

Root mass

170 mg

In New Zealand and North America, Zn is occasionally applied to nursery soils but not to pine plantations. In contrast, Australia has Zn-deficient soils (Armour et al. 1990; Boardman and McGuire 1990) and more than 55 tonnes of ZnSO<sub>4</sub> heptahydrate were applied to plantations in 2006 (May et al. 2009). Globally, several nursery trials have shown a positive response when applying Zn at rates less than 10 kg ha<sup>-1</sup> (Agnistikova and Scerbakov 1960; Tanaka et al. 1967; Bari and Gupta 1970; Knight 1976). In contrast, no growth benefit is expected when Zn fertilizers are applied to nondeficient seedlings. For example, seedlings in a greenhouse were not deficient and applying Zn-nitrate had no effect on early height growth (Figure 13).

### 11 Costs

Although treating seedbeds (in 1927) with 861 kg ha<sup>-1</sup> of commercial grade ZnSO<sub>4</sub> cost about \$303 ha<sup>-1</sup>, the reduction in hand weeding saved \$600 to \$1,200 ha<sup>-1</sup> (Wakeley 1927). Almost a century later the same zinc treatment would cost about \$4,300 ha<sup>-1</sup>. Since more effective herbicides, fungicides, and insecticides are available, some managers use Zn primarily as part of a sustainable production policy at a cost of only \$20 ha<sup>-1</sup> (at \$5 kg<sup>-1</sup> Zn). To keep application costs low, some managers add trace amounts of Zn to UAN solutions before spraying. However, a compatibility test is recommended before tank-mixing some Zn products with solutions that contain phosphate.

Table 3. A partial list of zinc fertilizers. G = Granular L = liquid.						
Name	Form	Common name	% Zn	% N	% S	Formula
Wolf Trax <sup>®</sup> DDP	G	Zinc oxide	62			ZnO
Brandt <sup>®</sup> Micronized	G	Zinc oxide	52		1	ZnO
Brandt <sup>®</sup> Seedzone™	L	Zinc oxide	40			ZnO
Sucra Min™	G	Zinc sucrate	36			ZnO -organic complex
Frit™ 317 G	G	Zinc oxide	36			ZnO - crushed glass
Brant <sup>®</sup>	G	Zinc sulphate	35		17	ZnSO <sub>4</sub> -H <sub>2</sub> O
Zeta Zinc 22™	G	Zinc sulphate	22		2	ZnSO <sub>4</sub> -7H <sub>2</sub> O
Tiger Micronutrients®	G	Zinc oxide	18		65	ZnO + S
CNI™	L	Zinc nitrate	17	7		Zn(NO <sub>3</sub> ) <sub>2</sub> -H <sub>2</sub> O
Liquid Zinc	L	Zinc sulphate	10	8	4	ZnSO <sub>4</sub> -H <sub>2</sub> O
Pro Zinc™ 10+	L	Zn EDTA	10	10		$NaC_{10}H_{12}N_2O_8NZn$
CM™ Liquid zinc	L	Zn lignin sulfonate	10		5	Zn - organic complex
Tracite ®	L	Zn lignin sulfonate	10		4	Zn - organic complex
Ultra-Che <sup>®</sup>	L	Zn diammonium	9	7		(NH4)2Zn-EDTA
Chelate solution	L	Zn EDTA	6	4		$NaC_{10}H_{12}N_2O_8NZn_2$
Brandt <sup>®</sup> EnzUP <sup>®</sup>	L	ZnEDTA	4			$NaC_{10}H_{12}N_2O_8NZn_2$
Epivio <sup>®</sup> Zn	L	Zn EDTA	2.9			$NaC_{10}H_{12}N_2O_8NZn_2$
Nutriculture <sup>®</sup> 12-2-12	G	Zn FDTA	0.05	12		$NaC_{10}H_{12}N_2O_{10}N7n_2$



Figure 13. In a greenhouse, applying Zn-nitrate increased Zn concentrations in needles (Blue), stems (Orange) and roots (black) of *Pinus taeda* seedlings (Zillmer 1978). Nine-month-old seedlings from the Indian Mound Nursery were transplanted into pots on January, 1978. Initially, concentrations in needles, stem and roots were 30, 38 and 25 μg g<sup>-1</sup> Zn, respectively. On May 22, (four weeks after fertilization), average values for needles, stem and roots were 24, 35 and 45 μg g<sup>-1</sup>, respectively. Seedling height growth (green) did not change after fertilization with zinc nitrate.

# **12** Conclusions

At nurseries with sufficient Zn in irrigation water, repeated harvesting of bareroot pine seedlings apparently has not lowered soil Zn (0-15 cm) to detrimental

levels. Inputs from irrigation, rain, dust, and phosphate fertilizers are typically sufficient to replace Zn removed during harvests. Most pine seedlings produced during the first eight decades of the 20<sup>th</sup> Century received no ZnSO<sub>4</sub>. The risk of a Zn deficiency is greatest for pine when nursery managers apply too much lime and do not irrigate seedlings. There is a risk of a Zn deficiency when non-mycorrhizal roots grow into recently leveled new ground.

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