Use of copper in pine nurseries

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Abstract

Copper has been used by nursery managers for more than 100 years to suppress fungi and as a fertilizer for more than 50 years. Consequently, nursery seedlings with copper deficiencies are rare, especially for broadleaf species. In many nurseries, soil contains <10 µg-Cu g^{-1} and in greenhouse trials, pine seedlings are relatively tolerant of soil levels with 35 µg-Cu g^{-1}. A million bareroot pine seedlings may contain 50 to 100 g-Cu and, when soil tests indicate low copper levels, managers might apply 1 kg-Cu per million seedlings. In contrast, it may take only 15 g-Cu to produce one million container-grown seedlings. Copper fertilization is typically not required when 30 cm of applied irrigation water contains 0.1 µg-Cu g^{-1} (supplying 0.3 kg-Cu ha^{-1}). This review highlights some of the past and current uses of copper in bareroot and container nurseries with a focus on deficiency and toxicity effects as well as the impact of various copper-based products and provides recommendations on ideal soil and foliar ranges.

Keywords

Nutrition; Foliar analysis; Soil testing; Hidden hunger; Toxicity

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Contents

1 Introduction

Copper (Cu^{++}) is an essential element for normal growth of seedlings (Shuman 1998; Landis and van Steenis 2000; Yruela 2015). Copper is used as a fungicide, a fertilizer and as a container-coating to prevent spiraling of pine roots (Burdett 1978; Ruehle 1985). This review will focus on use of copper in pine nurseries but will include experiments where hardwoods were grown in greenhouses and data from tree plantations. Most of the listed citations involve conifers since hardwood seedlings are seemingly less likely to develop copper deficiency (van den Burg 1983). Except for citrus, eucalypts and poplars, most copper deficiencies in hardwoods occur in experiments in greenhouses. Although numerous papers discuss use of copper in containers and deficiencies in agronomic crops, little has been published about deficiencies in pine seedbeds.

2 History

Research in the first quarter of the 20th century demonstrated that copper sulfate pentahydrate (CSP) could aid in controlling damping-off in bareroot nurseries (Hofmann 1914; Hartley 1915; Steven 1928). While conducting the tests, researchers noticed that high rates of CSP also suppressed weed growth (Kitchin 1920; Steven 1928). Due to costs and potential for buildup in the soil, use of CSP as an herbicide declined. However, managers continued to spray copper to control diseases (Vaartaja 1964; Miller and Roach 1980; Marx et al. 1984). In the USA, copper fungicides accounted for about 17% of the fungicides used on crops in 2008 (Fernandez-Cornejo et al. 2014).

Copper deficiencies were observed in conifer nurseries in England (1955), the Netherlands (1965) and New Zealand (1973) but symptoms were not noted in pine seedbeds in the USA. Although copper deficiencies occurred on ornamentals in retail container nurseries in Florida (Dickey 1965), bareroot nursery researchers were not aware of any copper deficiencies in North America (Anderson 1968; Stone 1968; Davey and Krause 1980). At that time, most soil laboratories did not routinely analyze copper levels (Iyer and Love 1974; Rathakette 1980; Youngberg 1984; Timmer 1985). Since a fertilizer test showed no statistically significant benefit to pines from applying copper on a pH 6.0 soil (Auten 1945), most managers in the USA did not see the need to apply copper fertilizers in bareroot nurseries.

Copper was mentioned once at a nursery-soils meeting in 1965 (Leaf 1965), but 15 years later target levels for copper were discussed (Abrahamson and Bickelhaupt 1980). Although iron and zinc fertilizers were used operationally, copper was not applied to container media (Baker 1957). Researchers created copper-deficient seedlings in greenhouses (Table 1) and deficiency symptoms were also observed in some fertilized pine plantations (Figure 1).

Figure 1. Copper deficient loblolly pine (*Pinus taeda* L.). This seedling was not copper deficient at planting but developed a deficiency in soil fertilized with nitrogen and phosphorus (P). Foliar samples taken from deficient seedlings in April, 2000 showed 0.1 and 0.0 µg-Cu g^{-1} while normal seedlings had 1.3 to 1.9 µg-Cu g^{-1} (South et al. 2004).

Prior to 1980, copper fertilizers were rarely applied operationally in pine nurseries in the USA. For example, as a precautionary measure in 1977, Jack May (May 1984) was likely the first in the South to suggest applying 1 kg-Cu ha^{-1} (33.6 kg ha^{-1} of Frit 503™) before sowing hardwoods. The following year, only one out of 32 nurseries applied trace elements to seedbeds (Marx et al. 1984). At that time, some laboratories provided tests for soil zinc (Youngberg 1984) but copper was only analyzed at the grower's request and several nursery manuals did not provide recommendations for when to apply copper fertilization (Stoeckeler and Jones 1965; Armson and Sadreika 1979; May 1984; Liegel and Venator 1987). Once private laboratories routinely tested soil copper (South and Davey 1983), then consultants used the precautionary principle and encouraged managers to apply CSP before sowing pine seed.

3 Soil tests

Copper in soils can be fixed, complexed, or soluble and "total" copper is the sum of these groups. The total far exceeds the portion that is soluble. In one example, the total amount extracted (using aqua regia) was 33 µg g^{-1} while the soluble portion (extracted using NH_4NO_3) was 0.1 µg g^{-1} (Siebe 1995). Although most of the copper in soils is unavailable or "fixed" (Yruela 2015), the portion that is "complexed" eventually becomes soluble and available to seedlings (Saur 1994). Most soil laboratories attempt to estimate the amount of copper in the soluble group. However, estimating the amount available to plants is not easy and fraught with variability.

Table 1. A selected list of photographs of copper deficiencies.

Species	Location	Photo on page	Reference
Betula alleghaniensis	Greenhouse	315	Bengtson 1968
Citrus spp.	Nursery	4	Hippler et al. 2017
Eucalyptus maculata	Field	261	Dell and Robinson 1993
Eucalyptus spp.	Field	78-86	Dell et al. 2001
Juglans nigra	Greenhouse	29	Hacskaylo et al. 1969
Larix leptolepis	Greenhouse	62	Baule and Fricker 1970
Liquidambar styraciflua	Greenhouse	30, 43	Hacskaylo et al. 1969
Nothofagus fusca	Greenhouse	65	Baule and Fricker 1970
Picea glauca	Greenhouse	46	Landis and van Steenis 2000
Picea glauca	Greenhouse	13	Landis et al. 1989
Picea sitchensis	Nursery	Plate 16-18	Benzian 1965
Picea sitchensis	Field	2657	Strullu and Bonneau 1978
Pinus radiata	Field	199	Ruiter 1969
Pinus radiata	Field	219	Will 1972
Pinus radiata	Nursery	211	Knight 1975
Pinus radiata	Nursery	96	Davis et al. 2015
Pinus radiata	Greenhouse	510	López Gorgé et al. 1985
Pinus sylvestris	Greenhouse	17335	Ivanov et al. 2016
Pinus taeda	Field	28	Jokela 2004
Pinus taeda	Field	387	South et al. 2004
Pseudotsuga menziesii	Nursery	150	Oldenkamp and Smilde 1966
Pseudotsuga menziesii	Field	76	van Goor 1970
Pseudotsuga menziesii	Field	63	Baule and Fricker 1970
Pseudotsuga menziesii	Field	342-343	Bonneau 1971
Pseudotsuga menziesii	Field	315	Bengtson 1968
Pseudotsuga menziesii	Field	2650	Strullu and Bonneau 1978
Pseudotsuga menziesii	Field	120	Carey et al. 1985
Populus spp.	Nursery	Plate 19-22	Benzian 1965
Populus deltoides	Greenhouse	29, 39	Hacskaylo et al. 1969
Robinia pseudoacacia	Greenhouse	29	Hacskaylo et al. 1969
Tectona grandis	Greenhouse	31, 33	Sujatha 2008
Tectona grandis	Greenhouse	195	Whittier 2018

Soluble estimates of copper obtained from weak extraction procedures might not related to the amount taken up by plants (Siebe 1995). "Available" copper can be estimated using various extraction methods: Mehlich 1, Mehlich 3, ammonium acetate (AA) and others (Davey 2002). The Mehlich 3 test is considered to be a "universal" soil extractant (Schroder et al. 2009). Extractions of identical soil samples might produce values of 2.9 (Mehlich 3), 2.1 (diethylenetriaminepentaacetic acid) and 1.5 (Mehlich 1) µg-Cu g^{-1} (Gartley et al. 2002; Mylavarapu et al. 2002; Wang et al. 2014).

Even when using the same extraction procedure, different laboratories will report different copper values for the same soil sample (White et al. 1980; Schroder et al. 2009; Santoro et al. 2017). As a result, one laboratory indicated the copper level in one sample was very high while two other laboratories indicated the level was low (Table 2). When using the Mehlich 3 extraction procedure, a ranking used for agronomic crops (and pine nurseries) is; 0.3, 0.8 and 0.9 µg-Cu g^{-1} for very low, low, and medium levels, respectively.

Table 2. Examples of copper soil test results (Mehlich 3) using three soil samples. Laboratory X indicated that soil A was very high (VH) while the other two laboratories indicated soil A was low (L) in copper. M = Medium level for soils.

	Laboratory		
Sample	X	Y	Z
	µg g^{-1}	µg g^{-1}	µg g^{-1}
Soil A	1.5 (VH)	0.7 (L)	0.49 (L)
Soil B	0.55 (M)	0.4 (L)	0.28 (L)
Soil C	0.35 (L)	0.4 (L)	0.23 (L)

4 Soils

Many soils in the United States are not deficient in copper. The average in acid mineral soils in Florida is 2.4 µg-Cu g^{-1} (Mehlich 3) (Mylavarapu et al. 2002) while silt loam soils in Arkansas average 5.3 µg-Cu g^{-1} (Bhandari and Ficklin 2009). Although most nursery soils contain less than 2.5 µg-Cu g^{-1} (Mehlich 1) (Tanaka et al. 1967; South and Davey 1983). The average level for 17 nurseries in the southern region of the United States was 1.4 µg-Cu g^{-1} (Mehlich 3) (Figure 2).

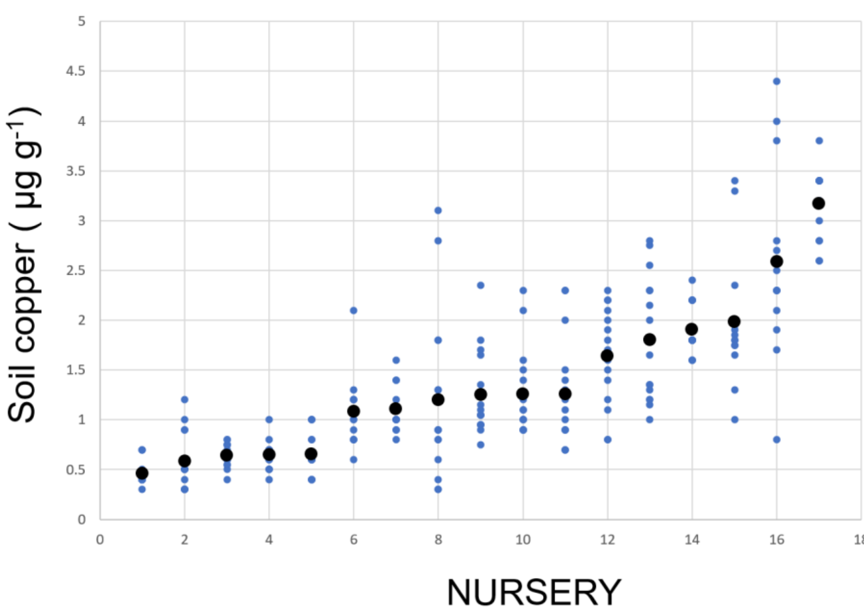

Figure 2. Soil copper (Mehlich 3) from 17 southern pine nurseries in the United States varies from a low of 0.2 µg-Cu g^{-1} to a high of 4.4 µg-Cu g^{-1}. Copper fertilizer was not applied at 10 of the nurseries. Each nursery is represented by a mean (black dot) of 15 soil samples (n = 255). In this survey, 21% of the samples were less than 0.7 µg-Cu g^{-1} and there were no reports of seedlings with a copper deficiency. Assuming one ha produces 1.7 million seedlings (and 21% of seedbeds are fertilized with 3.4 kg-Cu ha^{-1}) then approximately 460 kg-Cu are applied to produce a billion southern pine seedlings.

Pine trees typically do not exhibit copper deficiency symptoms when growing in non-fertilized soils and lower Coastal Plain soils typically have from 0.3 to 1.4 µg-Cu g^{-1}. The lowest value reported at a bareroot nursery is 0.1 µg-Cu g^{-1} (Mehlich 3) which is a common occurrence in sandy soils. Soil values in pine plantations rarely exceed 2.8 µg-Cu g^{-1} (Walker et al. 1989; NCSFNC 1992; Carlson et al. 2013). Since most of the copper ions are bound to organic matter, soil testing may provide a poor indication of levels available to the trees (Shuman 1991; Landis and van Steenis 2000). For example, when growing in 0.2 µg-Cu g^{-1} (Mehlich 3) soil, pine needles may have 3.4 µg-Cu g^{-1} (i.e. above average) (Carlson et al. 2013).

At nurseries established before 1960, higher than expected copper levels may reflect previous use of Bordeaux mixture for controlling fungi (Wakeley 1954; Verrall 1982; South and Davey 1983). Repeated use of Bordeaux mixture in vineyards and orchards increased copper levels to 200 to 500 µg-Cu g^{-1} (Alva 1993; Driscoll 2004; Jacobson et al. 2005). Due to air pollution, the level in soils in cities can exceed 20 µg-Cu g^{-1} (Fergusson and Stewart 1992; Yesilonis et al. 2008).

4.1 Soil acidity

For pines, copper deficiencies can occur in both acid and alkaline soils (Turvey and Grant 1990). Higher soil pH increases the strength by which copper is held by organic matter in solution (McLaren et al. 1990) and additional calcium reduces the amount of copper that remains soluble (Zhang et al. 2015). Therefore, lime reduces the amount of copper in solution (Massey 1972; Alloway 2008). The immobilization of copper in soils rich in $CaCO_3$ occurs by the precipitation of copper as copper carbonates (Garrido et al. 2005). However, in one greenhouse trial, increasing soil pH with $CaCO_3$ increased foliar copper by 1.5 µg g^{-1} (Table 3). Even when $CaCO_3$ levels are very high in nursery soils, pine needles can have 5-9 µg-Cu g^{-1} (Mexal and Fisher 1987; Landis 1988).

Table 3. The effect of lime (4,480 kg ha^{-1}) on total dry mass of red oak (*Quercus rubra* L.) seedlings (g) in greenhouse trials (Phares 1964). Seedlings in the 1962 trial were smaller than those grown in the 1963 trial. Overall, the lime ($CaCO_3$) reduced seedling mass by 17% and this resulted in a 40% increase in copper concentration (µg g^{-1}) in the leaves. The soil acidity before liming was pH 5.5-5.8. N=nitrogen; P=phosphorus; K=potassium; LSD = least significant difference; C.V. = coefficient of variation.

	Seedling mass		**Foliar copper**	
Year-Fertilizer	No lime	Lime	No lime	Lime
	g	g	µg g^{-1}	µg g^{-1}
1962-NP	13.86	10.12	4.3	6.8
1962-NPK	12.70	11.06	3.5	7.6
1962-NP	15.60	9.30	5	5
1962-NPK	13.59	10.81	6	6.8
1963-NP	14.57	17.00	3.5	3.5
1963-NPK	15.16	14.88	3.5	6
1963-NP	17.86	12.85	3.5	3.5
1963-NPK	16.13	12.22	1	3.5
Mean	14.9	12.3	3.8	5.3
Lime; P > F	0.005	--	0.021	--
LSD α=0.05	1.69	--	1.27	--
C.V.	11.4		25.5	

Fertilized loblolly pine (*Pinus taeda* L.) seedlings, growing in pH 3.9 soil, exhibited a copper deficiency (South et al. 2004). In theory, deficiencies might occur when soil pH is below 4.5 but the risk can be reduced with copper fertilization prior to sowing or with foliar application (Knight 1975). With foliar-applied copper, seedlings grown at pH 3.9 (soil at 0.2 µg-Cu g^{-1}) exhibited no signs of a copper deficiency (South et al. 2017). Good growth of pines can occur at pH 4.4 when needles contain 2.5 µg-Cu g^{-1} (MacDonald et al. 1986). Deficiencies in natural hardwood stands are rare (Table 1).

A negative correlation between soil pH and extractable copper can be achieved when testing a single soil type using lime in a laboratory or greenhouse (Massey 1972). In bareroot nurseries, a negative correlation may occur 30% of the time (Table 4). However, due to confounding factors (e.g. differences in soil types), there was no significant correlation between soil pH and soil copper in tree nurseries (South and Davey 1983; South et al. 2018), or in Florida soils (Mylavarapu et al. 2002), or in pine plantations (NCSFNC 1991).

Table 4. Examples of simple correlation coefficients between independent variables organic matter (OM) and soil acidity (pH) and dependent variable copper (Cu) for ten bareroot nurseries. At nurseries A, B and C, pH 5 soil might have 0.3 to 0.4 μg g^{-1} more Cu (Mehlich 3) than fields with pH 6 soil. At nursery D, the correlation indicates a 0.4 μg-Cu g^{-1} increase for every 1% increase in soil OM. Pearson correlation coefficients (r) in bold are significant (α = 0.07).

Nursery	pH	pH (r)	Soil copper (μg g^{-1})	Organic matter (%)	Organic matter (r)
A	4.7-6.1	**-0.544**	0.9-2.0	0.4-0.8	-0.196
B	4.2-6.0	**-0.370**	0.2-2.1	1.0-1.8	-0.100
C	5.1-6.3	**-0.341**	1.0-2.6	0.8-1.6	-0.211
D	4.0-5.2	-0.029	0.5-1.9	0.8-1.7	**0.285**
E	4.7-5.9	-0.022	0.6-3.2	0.5-1.3	0.004
F	4.6-5.7	0.034	0.9-2.4	0.4-1.0	-0.141
G	4.8-6.2	0.236	0.6-1.4	0.5-1.5	0.063
H	5.3-6.3	0.240	0.8-4.4	1.7-3.9	0.240
I	4.2-5.8	0.310	1.1-6.8	0.6-1.2	0.014
J	4.5-5.7	**0.422**	0.3-1.0	0.6-1.3	0.205

Low soil pH can increase the phytotoxicity of high rates of copper compounds (Gibson 1958; Bassett 1960). When treating soil with 34 kg-Cu ha^{-1}, germinating pine seedlings were injured at pH 3.9 but not at pH 5.6 (Figure 3).

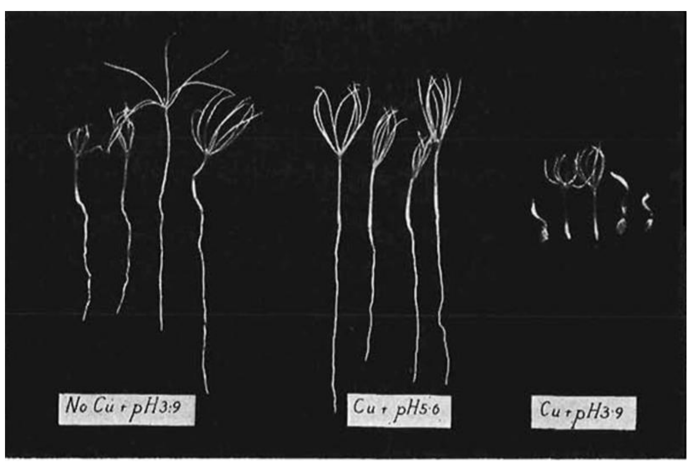

Figure 3. A pot study at Rotorua, New Zealand demonstrated an interaction between soil pH and treatment with copper oxychloride (photo from Bassett 1960). The copper fungicide caused no injury to pine at pH 5.6 (center) but injury occurred at pH 3.9 (right). Seven applications of copper oxychloride (34 kg copper oxychloride ha^{-1} per application) also injured *Pinus radiata* seedlings at the Kaikohe Nursery with a soil of pH 4.4 (Bassett 1960).

4.2 Organic matter

Mahler (2004) reported the likelihood of a copper deficiency is increased when soils contain more than 8% organic matter, but deficiencies have also occurred in soils with low organic matter. In New Zealand, deficiencies occurred in a pine nursery with 88 percent organic matter (Knight 1975). In England and Canada, copper deficiencies in nurseries occurred with 1.5 to 3 percent organic matter in soils (Benzian 1965, Teng and Timmer 1990). A copper deficiency has not yet been reported at pine nurseries with less than 0.8 percent organic matter (Tanaka et al. 1967; South and Davey 1983; South et al. 2018). These observations suggest that low soil organic matter is not a good predictor of a copper deficiency in 1-0 pine seedlings. Although significant correlations are unlikely at most bareroot nurseries, some nurseries have positive correlations (r= 0.28 and 0.43) for organic matter and soil copper (South et al. 2018; Table 4).

Some authors suggest micronutrient availability is enhanced with "ample organic matter" but, for copper, this is likely not true (Knight 1975; Siccama et al. 1980; Coleman et al. 1987, McLaren et al. 1990, NCSFNC 1992). Applying manure or sludge will increase copper levels in the soil (Korcak et al. 1979; Mexal and Fisher 1987) but adding peat or sawdust to pine seedbeds did not increase foliar copper in seedlings (Munson 1982; Mexal and Fisher 1987). In fact, at pH 7.4 these amendments likely reduced foliar copper levels by more than 50%. This is because the fixation of copper to organic matter is strong (Sauvé et al. 1997). Even though peat may contain 2.3 µg-Cu g^{-1}, no copper was present in the water extract from peat (Dumroese et al. 2018), which explains why a copper deficiency can occur on high peat soils (Knight 1975).

4.3 Copper in water

The need to fertilize with copper depends on the amount of copper applied in irrigation water. The risk of a copper deficiency is increased when irrigation water contains no copper (Landis et al. 2009) or when seedlings are grown without irrigation. Pine roots grow normally with 0.006 to 0.19 mg-Cu L^{-1} in greenhouses (Gorgé et al. 1985; Arduini et al. 1995) but they do not grow well when copper is absent (Goslin 1959). With water containing 0.03 mg-Cu L^{-1} (Parsons et al. 2001), irrigating with 126 ml/seedling/week (Oliet et al. 1999) for 40 weeks will supply 0.15 mg-Cu to each seedling. Likewise, applying 1 m of irrigation would add 0.1 kg-Cu ha^{-1} to a nursery soil when water contains 0.01 mg-Cu L^{-1} (Table 5). Irrigation water alone can produce pine seedlings with 4 to 12 µg-Cu g^{-1} in foliage (Walker and Huntt 1992; Hubbel et al. 2018). Copper fertilization is not required at nurseries that irrigate using water with 0.02 mg-Cu L^{-1} since 3.5 L will provide 0.07 mg-Cu per seedling (equivalent to 70 g per million seedlings). In some cases, distilled and deionized water contains 0.01 mg-Cu L^{-1} (Albano 2012) and small pine seedlings fertilized only with water can have 3 mg-Cu L^{-1} in shoots (Gruhn 1989).

Rainfall typically has less than 0.001 mg L^{-1} of copper but in a few cases the amount may be 0.003 mg L^{-1} (Bales et al. 1999). Rural streams may contain 0.002 to 0.029 mg L^{-1} (Aubertin et al. 1973; Caldwell 1992; Silapajarn and Boyd 2005) and urban streams may contain more than 10 mg L^{-1} (Bodo 1989; Bales et al. 1999). Copper in tap water may be >0.1 mg L^{-1} (Domek et al. 1984; Wang et al. 2014) and sometimes old buildings will contain >0.25 mg L^{-1} (Wang et al. 2014). The national threshold for drinking water in the United States is 1.3 mg L^{-1} of copper.

Table 5. The amount of copper added to pine seedbeds depends on the amount of irrigation applied and the concentration of copper in the irrigation water. When the level of copper in irrigation water is 0.01 mg L^{-1}, then 60 cm of irrigation would add approximately 60 g-Cu ha^{-1} yr^{-1} or 0.012 mg per seedling (at a container tray density of 500 cells per m^2). About 14% of irrigation water samples from southern pine nurseries contain less than 0.01 copper but 26% contain 0.01 to 0.02 mg-Cu L^{-1} and 60% contain 0.03 to 0.08 mg-Cu L^{-1}.

	Irrigation water copper (mg L^{-1})			
Irrigation level over 6 months	0.01	0.01	0.03	0.08
	mg $seedling^{-1}$	g ha^{-1}	g ha^{-1}	g ha^{-1}
10 cm	0.002	10	30	80
30 cm	0.006	30	90	240
60 cm	0.012	60	180	480
1 m	0.020	100	300	800

4.4 Mycorrhiza

In most soils, roots can take up a sufficient amount of copper so that non-mycorrhizal seedlings do not become deficient in copper (Gildon and Tinker 1983; Jones and Hutchinson 1986; Cumming and Weinstein 1990; Adriaensen et al. 2005; Walker and McLaughlin 1997; van Tichelen et al. 1999; Quoreshi and Khasa 2008; Wen et al. 2016; Anwar et al. 2019). For example, stunted, non-mycorrhizal loblolly pine seedlings exhibited P deficiency symptoms and yet had 3 to 5 µg-Cu g^{-1} in shoots (Table 6). Similar foliar copper concentrations were observed for ectomycorrhizal and non-mycorrhizal seedlings (South et al. 2018) and for seedlings growing in fumigated and non-fumigated soil (Danielson 1966). Mycorrhizae limits the amount of copper found in needles (Gruhn 1989; Adriaensen et al. 2005) which may explain why copper concentrations in needles stay about the same as soil copper levels increase.

Table 6. The presence of ectomycorrhiza increased copper concentrations (µg g^{-1}) in shoots and roots of loblolly pine (*Pinus taeda* L.) at a bareroot nursery in Alabama. Normal seedlings (0.68 g dry mass) were mycorrhizal while stunted seedlings (0.20 g dry mass) were non-mycorrhizal. Seeds were sown on fumigated "new ground" on April 9th (South et al. 1988) and seedlings were sampled on July 29, 1986. LSD = least significant difference; C.V. = coefficient of variation.

Sample location	Normal shoot	Stunted shoot	Normal root	Stunted root	Normal shoot	Stunted shoot
	Cu µg g^{-1}	Cu µg g^{-1}	Cu µg g^{-1}	Cu µg g^{-1}	P µg g^{-1}	P µg g^{-1}
1	9	5	17	16	0.15	0.09
2	8	4	17	13	0.16	0.06
3	8	4	16	10	0.15	0.06
4	9	3	13	6	0.15	0.06
5	7	4	12	7	0.14	0.07
Mean	8.2	4.0	15.0	10.4	0.15	0.068
P > F	0.001	--	0.011	--	0.001	--
LSD α=0.05	1.4	--	2.9	--	0.02	--
C.V.	12.7	--	12.8	--	10.7	--

4.5 Phosphorus

Fertilization with P can induce a copper deficiency in conifers (Oldenkamp and Smilde 1966; Ruiter 1969; Carey et al. 1985; Saur 1993). When pines were grown in non-limed soil, P fertilization reduced copper concentrations in needles by 1 to 9 µg g^{-1}

(Pritchett and Llewellyn 1966; van Lear and Smith 1972; Smilde 1973; Saur 1994; Figure 4). In one greenhouse trial, fertilizing a sandy soil with monocalcium phosphate and urea resulted in pine needles with only 1.3 µg-Cu g^{-1} (van Lear and Smith 1972). On alkaline soils, a copper deficiency can be induced by fertilizing hardwoods with high rates of P (Timmer and Leyden 1980; Lambert 1982; Teng and Timmer 1990). Similar results occur when P is applied to organic soils in Florida (Forsee and Allison 1944).

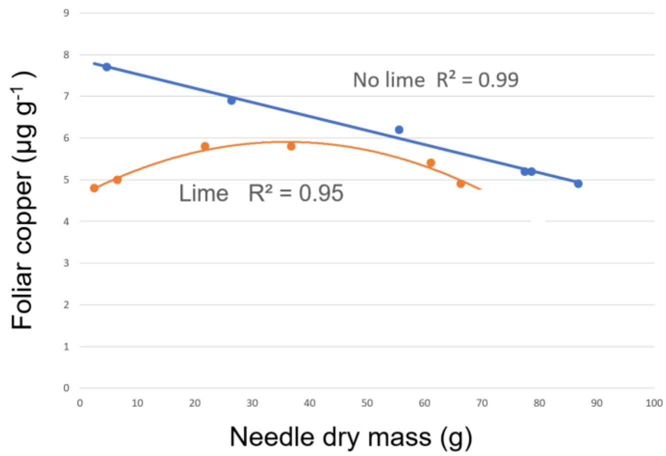

Figure 4. When 1-0 Scots pine (*Pinus sylvestris* L.) seedlings (5 per pot) were grown outside in pots containing 11 kg of soil with no lime (blue line), the concentration in foliage (µg-Cu g^{-1}) declined as seedling size increased (Smilde 1973). Smallest seedlings received 25 µg-P g^{-1} (as reagent grade calcium phosphate) while largest seedlings received 375 µg-P g^{-1}. In contrast, the application of lime (CaO orange line) reduced the growth of pine seedlings and also lowered copper levels in the needles. When treated with the high rate of calcium phosphate, the lime treatment reduced seedling biomass by 26%. The application of lime increased soil $pH_{(KCl)}$ to 4.3 (control = pH 3.9).

4.6 Nitrogen

The rate of nitrogen (N) fertilization may not affect foliar copper concentration of pine seedlings (MacDonald et al.1986; Olykan and Adam 1995; Schmidtling 1995; McGuire 1998; Warren and Adams 2002) or Douglas fir (*Pseudotsuga menziesii* (Mirbel) Franco) (Birchler et al. 2001). Although applying 600 kg N ha^{-1} at a bareroot nursery did not induce a copper deficiency in pine seedlings (Dierauf 1991), irrigating pine seedlings with nitric acid (and sulfuric acid) can decrease uptake of copper (Walker and McLaughlin 1997).

Although N can reduce foliar copper concentrations of some hardwood species (Kramer 2008; Wooldridge et al. 2009) it did not affect foliar concentrations in red oak (*Quercus rubra* L.) (Phares 1964), pin oak (*Quercus palustris* Münchh.) (Ponder et al. 2008) and teak (*Tectona grandis* L.f.) (Whittier 2018). Sometimes fertilization of hardwoods with low rates of ammonium nitrate will increase the uptake of copper (van den Burg 1983).

4.7 Lime

Applying lime will reduce the growth of certain conifers (de Vries 1963; Smilde 1973; Wall 1978; Coultas et al. 1991; Helm and Kuser 1991; Altland and Jeong 2016; South 2017; Anwar et al. 2019) and sometimes lime will reduce available soil copper (Garrido et al. 2005; Pardo et al. 2014). It is possible that adding lime to container media has resulted in copper deficiencies of pine seedlings (Dumroese et al. 1990). For example, adding dolomitic lime to a peat:vermiculite:sand medium reduced concentrations in pine needles by about 2 µg-Cu g^{-1} per unit increase in pH (Helm and Kuser 1991). On contaminated soils, adding lime will reduce copper toxicity symptoms (Reuther et al. 1953; Verdugo et al. 2010) and on acid, copper deficient soils, adding dolomitic lime can reduce deficiency symptoms and increased growth of pines (Figure 5).

Figure 5. Loblolly pine (*Pinus taeda* L.) growing in research trial established on a Mascotte soil in Pierce County, Georgia (South et al. 2004). Plots in one treatment (right photo) were treated with dolomitic lime (6.7 Mg ha^{-1}) and copper sulfate (36 kg ha^{-1}) on November 16 and seedlings were planted a month later on December 15, 2000 (photos taken 13 December, 2002). The photo on the left shows copper deficient pines growing on no-lime plots (soil with 0.0 to 0.7 µg-Cu g^{-1} Mehlich 1). All plots in the entire area were fertilized with diammonium phosphate (280 kg per ha) on August 30, 2001 which caused a copper deficiency on low pH soil. The unlimed soil (left photo) was pH 3.9 and the limed soil (right photo) was pH 4.9.

Adding dolomitic lime (3.2 Mg ha^{-1}) at one nursery had no effect on foliar copper concentrations of pine seedlings (South et al. 2017) but in greenhouse trials, adding lime lowered the concentration in pine needles (Smilde 1973; Plass 1969). When dolomitic lime decreases growth of pine seedlings, the foliage levels may remain the same (Figure 6) or they might increase by 3 to 4 µg-Cu g^{-1} (Coultas et al. 1991).

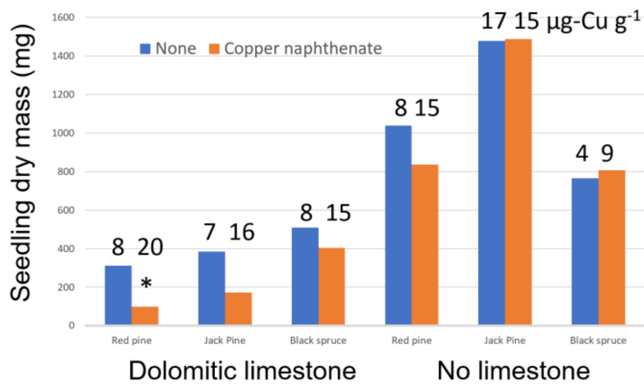

Figure 6. The effect of copper naphthenate (wood treatment) and dolomitic limestone on growth of 7-month-old seedlings growing in sphagnum peat (Wall 1978). All seedlings less than 600 mg were growing in media amended with 10 g of dolomitic limestone per liter of peat. In this trial, seedlings grew better at pH 4.2 (unlimed peat) than at pH 5.8 (limed peat). Painting wooden flats with copper naphthenate caused a significant (α=0.05; *) reduction in growth of red pine (*Pinus resinosa* Aiton) seedlings with roots in limed peat. Values above bars indicate copper concentration of shoots at harvest. For black spruce [*Picea mariana* (Mill.)], the copper naphthenate treatment increased foliar levels of copper (α=0.05). Statistical tests for foliar levels were not conducted for red pine and jack pine (*Pinus banksiana* Lamb.).

5 Copper in fertilizers

Diammonium phosphate may contain 1 to 41 µg-Cu g^{-1} (Raven and Loeppert 1997) and dolomitic lime can contain 8 µg-Cu g^{-1} (Shuman 1986). When pine seedlings are fertilized with a slow-release fertilizer that contains no copper, foliage may contain only 0.4 to 0.5 µg-Cu g^{-1} at harvest (South et al. 2005). However, other products add enough so that 200 kg-N ha^{-1} would supply 0.66 kg-Cu ha^{-1} (Blythe et al. 2006; Jacobs and Landis 2014).

Various organic fertilizers can be relatively high in copper. Applying 10,000 kg ha^{-1} of chicken manure might add 1.6 kg-Cu ha^{-1} (Davis et al. 2006) while the same amount of sewage sludge might add 11.5 kg-Cu ha^{-1} (Rose et al. 1995). Although sewage sludge was tested at pine nurseries in the past, most nursery managers would rather apply fertilizers that have a reliable composition from year to year (Aldhous and Mason 1994).

6 Copper in fungicides

Copper-based fungicides have been used operationally in pine nurseries (Wakeley 1954; USFS 1991, Karmiłowicz 2019). Overuse of copper-fungicides in some citrus orchards increased soil copper to toxic levels (Arias et al. 2004; Driscoll 2004). In contrast, managers rely on newer fungicides to control diseases in pine nurseries (McCain and Smith 1978; South and Zwolinski 1996; Sorvari and Jaakkonen 2011; Keča 2016) and therefore, unless located near cities, copper in USA nursery soils do not exceed 10 µg g^{-1} (Mehlich 3).

Pine plantations in Australia are fertilized with about 1.2 kg copper ha^{-1} (mid-rotation) for a nation-wide total of about 18,500 kg-Cu yr^{-1} (May et al. 2009). In the USA, it is estimated that about 460 kg of copper are used to produce a billion bareroot pine seedlings. As a comparison, 6.2 million kg of copper fungicides were applied to agronomic crops in 1997 (Gianessi and Marcelli 2000).

7 Copper removed at harvest

The amount of copper removed by harvesting bareroot seedlings depends on species, cultural practices, seedling age and copper in irrigation water. Bareroot pine seedlings may contain 50 to 100 g of copper per million seedlings (Knight 1978; Boyer and South 1985; Pritchett and Fisher 1987) and a million container-grown pines might contain 60 g-Cu (Mitchell et al. 1990; Olykan and Adam 1995; McGuire 1998). Harvesting bareroot hardwood seedlings may remove about 200 to 300 g-Cu ha^{-1} or 300 to 690 g-Cu per million seedlings (Arnold and Struve 1993; dos Santos 2006). In comparison, harvesting corn grain removes about 30 g-Cu ha^{-1} (Heckman et al. 2003) and harvesting 16-year-old pine trees may remove about 310 g-Cu ha^{-1} (Muyambo 2017). Total copper levels in topsoil will decline over time when harvest rates exceed inputs from irrigation, rainfall, and fertilizers. At one nursery, the annual net loss (Mehlich 3) was estimated to be 450 g-Cu ha^{-1} (Figure 7).

The distribution of copper in seedlings is not uniform. In a bareroot nursery, the concentration in roots can be 50-200% higher than the concentration in foliage (Goslin 1959; Danielson 1966; Stone and Timmer 1975; Knight 1978; Boyer and South 1985; Pritchett and Fisher 1987; McGuire 1998; Saur 1990; Saur 1993; Tsakaldimi and Ganatsas 2006; Potvin et al. 2014; Zong et al. 2015). Therefore, an underestimation (of copper removed at harvest) will occur if one assumes the entire seedling has the same copper concentration as the foliage.

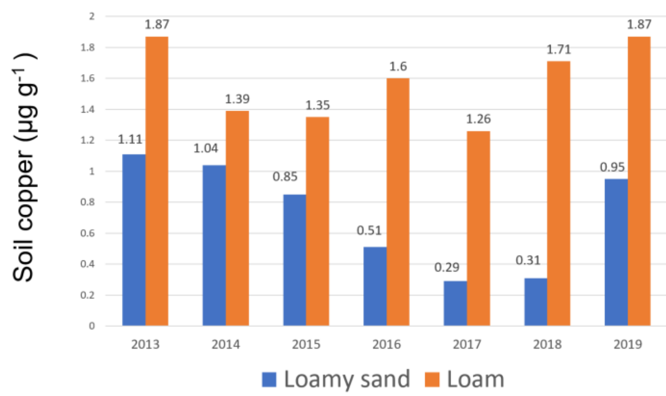

Figure 7. At some pine nurseries, the level of soil copper (Mehlich 3) gradually declines over time as harvesting seedling crops removes copper. At one loamy sand nursery the decline was about 0.2 µg g^{-1} $year^{-1}$ and the 0.6 µg g^{-1} increase observed in 2019 was due, in part, to applying 0.8 kg-Cu ha^{-1} (copper sulfate chelated with citric acid). At the loam nursery, copper was not applied as a fertilizer (1989 to 2019) and annual inputs and outputs of available copper appear to average out on a decadal basis.

8 Copper deficiency

Copper deficiency occurs when pine seedlings show visual symptoms (Figure 1) or when growth is limited by insufficient copper (i.e. hidden hunger). A copper deficiency should not be defined solely by tissue analysis. For example, Cu-contaminated foliage with 6 µg-Cu g^{-1} can exhibit visual deficiency symptoms (Teng and Timmer 1990) and symptomless pines can grow well with 1.4 µg-Cu g^{-1} in the foliage (van Lear and Smith 1972; Sypert 2006). Also, vector analysis (Teng and Timmer 1990, Timmer 1991) is not useful for identifying a copper deficiency (Valentine and Allen 1990;

Mead and Mansur 1993; Sypert 2006; Tsakaldimi and Ganatsas 2006). For example, since vector theory is independent of traditional critical foliar values (Timmer 1991), seedlings with 10 µg-Cu g^{-1} in needles could be declared Cu-deficient when soil fumigation increases both foliar copper concentration (15 µg-Cu g^{-1}) and seedling growth (Danielson 1966). In some cases, interpretations using vector analysis are made without analyzing foliar levels of copper (Timmer 1985).

Genetics affects copper deficiency and plants may have high, medium or low sensitivity to a copper deficiency (Alloway 2008). The more sensitive tree species include citrus, larch (*Larix kaempferi* (Lam.) Carrière) and Douglas fir, while Norway spruce (*Picea abies* (L.) H. Karst.) and Scots pine (*Pinus sylvestris* L.) are insensitive (Carey et al. 1985; Turvey and Grant 1990). Deficiency symptoms were not noticed when needles contained 0.4 µg-Cu g^{-1} (South et al. 2005) or trace amounts (Slaton and Iyer 1974) which suggests that pine is a low sensitive genus.

Copper deficiencies in seedlings can occur in greenhouses (van den Burg 1983; Majid 1984; Gherardi et al. 1999; Landis and van Steenis 2000), on certain sandy nursery soils (Benzian and Warren 1956; Oldenkamp and Smilde 1966; Knight 1975) and in fertilized plantations (van Goor 1970; Carey et al. 1985; Simpson and Grant 1991; South et al. 2004). Although copper deficiencies have occurred in container nurseries (Dickey 1965; Dumroese et al. 1990; Landis and van Steenis 2000), they have not been recorded for bareroot nurseries in the eastern USA. In Wisconsin, no visual deficiency symptoms occurred when 3-year-old pine seedlings contained trace amounts of copper in the foliage (Slaton and Iyer 1974) and no reported visual symptoms occurred when foliage contained 0.4 to 0.6 µg-Cu g^{-1} (van Lear and Smith 1972; South et al. 2005). Although photos exist from Europe (Table 1), there are no published photographs of copper-deficient seedlings in bareroot nurseries in North America.

Figure 8. The glass container on the right contains three loblolly pine (*Pinus taeda* L.) seedlings (normal color Munsell 7.5 GY 5/6) growing in nutrient solutions with copper chloride. The nutrient solution in the left container does not contain copper and one seedling exhibits twisted growth. Copper-deficient needles also had a normal color but the dead ends of needles had a color of 5.0 YR (Lyle 1969).

Symptoms of copper deficiency in conifers include plagiotropic growth (Figure 1), thin and flaccid needles (Figure 8), twisted needles (Figure 9) and brown-dead needle tips. Deficiencies have been reported for pine plantations in Australia (Simpson and Grant 1991), New Zealand (Hunter et al. 1990), France (Strullu and Bonneau 1978; Saur 1994) and the United States (South et al. 2004; Vogel and Jokela 2011). In North America, the most likely regions for a copper deficiency include the Great-Plains Region, Florida, and coastal regions of Georgia, South Carolina and North Carolina (Kubota 1983; Holmgren et al. 1993). In Alabama, copper has not been found to be deficient for any crop (Sparr 1970, Mitchell and Huluka 2012). Non-fertilized pine seedlings (1.9 mm diameter) growing in pots containing a Lakeland sand (0.3 µg-Cu g^{-1} (AA)) had 17 µg-Cu g^{-1} in the shoots (van Lear and Smith 1972) which is not considered deficient. Pine seedlings low in copper at time of lifting (0.5 µg-Cu g^{-1}) can grow well after planting (South et al. 2005).

Figure 9. Twisted needles on larch (*Larix kaempferi* (Lam.) Carrière) seedlings at the Abbots Moss Nursery near Cheshire, England 2011. Soil in block A2 was pH 5.0 $_{\text{(water)}}$, soil organic matter 3.9%, 93 µg-P g^{-1}, 284 µg-Ca g^{-1}, and soil copper 0.05 µg-Cu g^{-1} (photo by Alex Steepe – Forestry England). On Sitka spruce (*Picea sitchensis* (Bong.) Carr.), twisted needles with "needle tip-burn" usually developed suddenly during dry hot spells and was rarely seen in seedbeds before August (Benzian 1965; Aldhous 1972). Larch may be an indicator species since it shows deficiency symptoms sooner than hardwoods or Douglas fir (*Pseudotsuga menziesii* (Mirbel) Franco) (van den Burg 1983).

Bareroot seedlings that appear normal but increase in growth after copper fertilization might have a "hidden hunger" for copper. Examples of "hidden hunger" exist for container-grown seedlings (Dell 1994; Arnold et al. 1997; Aldrete et al. 2002), but examples from bareroot nurseries are difficult to prove. For example, increasing the soil by 25 µg-Cu g^{-1} might result in a non-statistical increase in pine seedling biomass of

27% (Kukkola et al. 2000). Even when seedlings are growing in artificial media, it may be difficult to show any growth benefit from fertilizing with copper chloride (Hacskaylo et al. 1969) or CSP (Smith 1943; Lozano and Morrison 1982; van den Driessche 1989; Gherardi et al. 1999).

Sulfur is confounded with copper when fertilizing with CSP and, as a result, it can be difficult to prove a hidden hunger. For example, on limed soil, a CSP treatment increased height of pine seedlings by 32 cm but it added both copper (9 kg ha^{-1}) and sulfur (4.5 kg ha^{-1}) to the soil (South et al. 2004). Therefore, it is recommended that cupric carbonate and zinc sulfate treatments be included in tests designed to prove a hidden hunger response. However, since copper is also a fungicide, some might assume additional growth was due to a reduction in pathogens (Kais 1975; Woollons and Hayward 1984; Korthals et al. 1996).

Most nursery trials have fewer than five replications and therefore the statistical power of the test is low (VanderSchaaf et al. 2003; South and VanderSchaaf 2017). As a result, in some trials (Auten 1945; Heale and Ormrod 1982) a 200 percent increase in shoot biomass was not statistically significant (α = 0.05). In a trial with three replications, statistical methods were unable to demonstrate an increase of 20 µg-Cu g^{-1} was not due to chance (Oliet et al. 1999). Typically, greenhouse trials (using 100% sand and deionized water) are required to graphically illustrate the "hidden hunger" zone for copper.

9 Copper toxicity

Jack May realized that applying too much copper could injure pine seedlings (Figure 10), and he may have been the first in the South to request a nursery soil be tested for copper (letter dated December 21, 1973). When growing in soil, copper is more toxic to pine seedlings than zinc, iron, or manganese (van Lear and Smith 1972) and too much copper can injure roots (Figure 11). When grown in pots containing perlite and high levels of CSP, pine seedlings may have 20 µg-Cu g^{-1} in needles (Adriaensen et al. 2005). However, when growing in soil, a few reports indicate concentrations in pine needles can exceed 50 µg-Cu g^{-1} (Lombardo et al. 2001; Nieminen et al. 2004; Selivanovskaya and Latypova 2006). In trials where seedlings were treated three times with cupric oxide, phytotoxicity was observed when needles contained more than 75 µg-Cu g^{-1} (Fraser et al. 2019). After deficient seedlings are sprayed with copper, foliar tests of healthy seedlings may show 107 µg-Cu g^{-1} (Knight 1975). In nurseries, the toxicity risk to bareroot seedlings is minimal because managers apply low rates of copper and manure is typically not added to fallow land.

Pine seedlings can be used to restore sites that have been contaminated with copper. In some cases, roots are not injured until levels in roots exceeds 160 µg-Cu g^{-1} (Fuentes et al. 2007b; Jeyakumar et al. 2014). Pine seedlings are tolerant of 4 kg-Cu ha^{-1} applied before sowing or even a month after the summer solstice when primary needles are treated with CSP. Even when applied after sowing in mid-May, CSP at 62.5 kg-Cu ha^{-1} did not injure pine seedlings (Neuwinger and Schinner 1980). Pine seedlings transplanted into copper-contaminated soil can survive and grow (Jeyakumar et al. 2014: Zong et al. 2015). Pine seedlings tolerate copper better than hardwoods (Heale and Ormrod 1982; Fuentes et al. 2007b).

At some hardwood nurseries, chelated copper is applied in the fall to induce early defoliation (Bi et al. 2005). Assuming 200 l ha^{-1} of solution was applied on 3

October, a 2.1% solution (4.2 kg CuEDTA ha^{-1}) resulted in 86% defoliation of apple (*Malus domestica* Borkh.) seedlings by November 5th (Knight 1983). Due to possible phytotoxicity, some product labels do not recommend applying 1.5 kg Cu-EDTA ha^{-1} to foliage in greenhouses.

Some toxicity trials are unclear since they specify a solution concentration of copper but not the amount to apply per area. Applying a 0.25% copper solution at 40 L/plot will apply twice as much copper as spraying 10 L/plot with a 0.5% solution. One product label indicates that spraying to run-off requires 930 L ha^{-1} or more but it can sometimes exceed 9,000 L ha^{-1}. A 0.5% solution at these two rates would range from 4.6 to 45 kg-Cu ha^{-1}. It is false logic to assume the solution concentration is the only factor that affects copper toxicity in nurseries.

Figure 10. Effect of a high rate of copper carbonate on loblolly pine (*Pinus taeda* L.) seedlings at the Ashe Nursery (Brooklyn, Mississippi). Seedbed photo taken in February 1971 by Jack May. The treatment was likely part of a weed control study

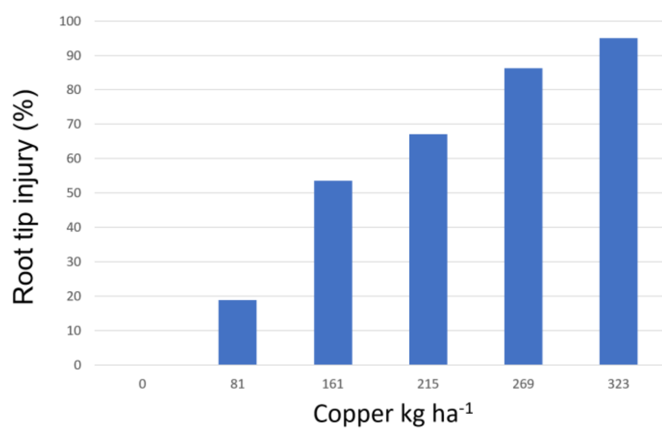

Figure 11. The effect of copper sulfate pentahydrate (copper sulfate pentahydrate = mass of copper x 4) on root injury in seedbeds of western white pine (*Pinus monticola* Douglas ex D. Don) (Wahlenberg 1930). The 269 kg-Cu ha^{-1} treatment slowed the rate of germination but did not affect total germination of pine seed. The 81 kg-Cu ha^{-1} rate (i.e. 324 kg of copper sulfate pentahydrate) can cost more than $700 (Goodwin and Straus 2006). When soil tests are low, managers in the southern United States apply less than 4 kg-Cu ha^{-1} before sowing.

10 Tissue analysis

Although tissue analysis has been used to determine if seedlings are deficient in copper (Kruger 1967; Knight 1975; Dumroese et al. 1990; South et al. 2004; Coburn and Moreno 2007), critical values are not known with any exactness (Jokela 2004). Estimates for the minimum foliar value for conifer needles include: 4 µg g^{-1} (Timmer 1991; Landis et al. 2005), 3 µg g^{-1} (Lambert and Weidensaul 1982; May 1984; Sypert 2006), 2 µg g^{-1} (Knight 1978; Majid 1984; Jokela 2004; Carlson et al. 2013), 1.5 µg g^{-1} (Jokela 2004) and 1 µg g^{-1} (Simpson and Osborne 1993; Sayer et al. 2009). Deficient pine seedlings tend to have needles with less than 2 µg-Cu g^{-1} (Ruiter 1969; White and Mead 1971; Knight 1975; Lambert and Weidensaul 1982; Gorgé et al. 1985; Hunter et al. 1990; Miller 1990; South et al. 2004; Fuentes et al. 2007a).

Due to carbohydrate dilution (Saur et al. 1995; Starkey and Enebak 2012), copper in foliage of bareroot loblolly pine seedlings can drop from 10 µg-Cu g^{-1} (August) to 4 µg-Cu g^{-1} (September) in just one month. At the end of the growing season, bareroot pine needles can range from 2 to 19 µg-Cu g^{-1} (Danielson 1966; Flinn et al. 1980; Boyer and South 1985; McGuire 1998) while 5 to 16-year old pines may range from 2.0 to 3.3 µg-Cu g^{-1} (White and Mead 1971; Helmisaari 2008; Carlson et al. 2013; Muyambo 2017). Typically for pines, higher values are associated with primary needles while older and denser secondary needles from 3-year-old seedlings have lower values. Foliage of 6-month-old longleaf pine seedlings may range from 8 to 27 µg-Cu g^{-1} (McGuire 1998; Starkey and Enebak 2012) but sometimes it can be as low as 0.4 µg-Cu g^{-1} without showing deficiency symptoms (South et al. 2005).

The likely reason the median copper value of bareroot loblolly pine seedlings was higher in 2010 (13.5 µg-Cu g^{-1}) than in 1982 (6 µg-Cu g^{-1}) was due to foliar applications of copper before sampling (Boyer and South 1985; Starkey and Enebak 2012; Rolando et al. 2019). Several studies show seedlings treated with foliar applications of copper test much higher than untreated seedlings (Iyer and Wilde 1974; Knight 1975; Majid and Ballard 1990; Close et al. 2003; Rolando et al. 2017). Once foliage has been sprayed with copper, then surface residues can make a "not deficient" diagnosis incorrect (Teng and Timmer 1990). Sometimes epicuticular wax can have ten times as much copper as de-waxed needles (Rautio and Huttunen 2003).

Although tissue analyses are recommended for determining fertilizer needs for container-grown seedlings (Landis and van Steenis 2000; Coburn and Moreno 2007), managers of bareroot nurseries rely on soil tests, which typically cost $10 less per sample. This is because rates for pre-sowing fertilizer applications are not based on foliage tests from the previous cover-crop. However, soil testing may provide a poor indication of copper availability (Iyer and Wilde 1974; Shuman 1991; Landis and van Steenis 2000). For example, roots growing in 0.3 µg-Cu g^{-1} soil (AA) can produce pine needles with 17 µg-Cu g^{-1} (van Lear and Smith 1972). In general, there is not a good correlation between available copper in the soil and copper content of conifer needles (Stone and Timmer 1975; Saur 1990; Nieminen et al. 2004; Figure 12).

Sometimes foliar analyses show no difference in copper concentration between normal and twisted needles (Dumroese et al. 1990; Saur et al. 1995). Therefore, analyses of stems (Nieminen et al. 2004) or roots (Stone and Timmer 1975; Saur 1990) might be a more discriminating method to detect the copper status of seedlings. At other times deficient seedlings have higher copper levels due to the Steenbjerg effect

(Turvey and Grant 1990). Therefore, some conclusions (based only on foliage tests) about the cause of twisted needles were wrong.

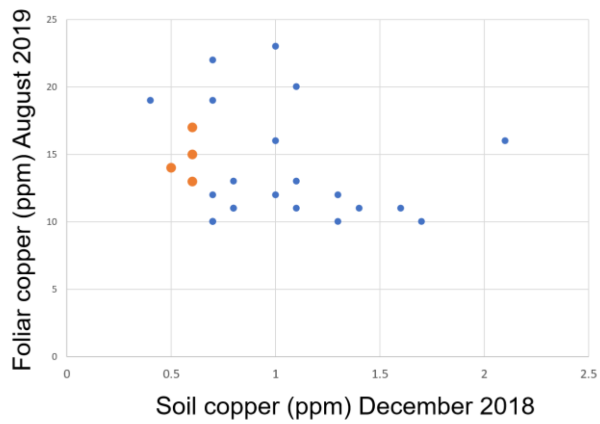

Figure 12. Soil sampling (n=26) at a sandy loblolly pine (*Pinus taeda* L.) nursery indicates no relationship between copper in the shoot in August 2019 (3 months after sowing) and soil copper (Mehlich 3) in December 2018. Soil tests indicate treating four fields (orange dots) with 3.36 kg ha^{-1} of copper in March 2019 increased soil copper by 1.5 µg g^{-1} (data not shown). Fields not treated with copper (blue dots) averaged 1.0 µg-Cu g^{-1} in December 2018 and these fields averaged 1.2 µg-Cu g^{-1} eleven months later.

11 Recommendations

The “adequate” range for pine foliage (3-20 µg-Cu g^{-1}) is the same for both container and bareroot seedlings. Even so, the amount of copper fertilizer recommended to produce one million pine seedlings is much higher for bareroot stock. Some suggest fertilizing bareroot seedlings with 1 kg-Cu per million seedlings (perhaps 1.7 kg-Cu ha^{-1}) while 16 g-Cu is sometimes applied to one million container-grown pine seedlings (Zhu et al. 2019). Tradition is the main reason behind the large difference in fertilization rates.

11.1 Bareroot

There are four basic approaches to fertilizing bareroot pine seedlings with micronutrients: (1) wait until visual symptoms appear (Figure 9) and then spray fertilizer over seedlings with twisted foliage (Benzian and Warren 1956; South et al. 2004; Mitchell and Huluka 2012); (2) test foliage in June and apply fertilizer to deficient seedlings after reviewing results; (3) routinely apply low rates of micronutrients to foliage as a preventative measure (Iyer and Wilde 1974; Landis et al. 1989); and (4) apply micronutrients to soil or foliage if soil tests are at a low level. Prior to 1980, millions of bareroot seedlings were produced using method #1 without any published reports of copper deficiency (Rathakette 1980; Hopmans and Flinn 1983). Method #2 is rarely used since the cost of 31 foliage samples can exceed $800 (Landis et al. 2005). Method #3 is used when managers apply trace elements in complete fertilizers (South 2019). Method #4 involves applying 2 to 3.4 kg-Cu ha^{-1} when soil value is $\leq$0.7 µg-Cu g^{-1} (Aldhous 1972; Davey and McNabb 2019). However, a few authors suggest applying higher rates of 5 to 10 kg-Cu ha^{-1} (Maxwell 1988; Turvey and Grant 1990; Aldhous and Mason 1994). Although doubling the application rate will double the cost, it might also lengthen the interval between treatments (Korcak et al. 1979).

To avoid a buildup in soil copper, the recommendation in Europe is to not apply more than 28 kg-Cu ha^{-1} over a 7-year period. At most sandy nursery seedbeds in the USA, the total amount applied over an 8-year period does not exceed 8 kg of elemental copper. For example, nurseries on a schedule of two pine crops followed by two cover-crops, might apply 3.4 kg-Cu ha^{-1} in February of the first and fifth years. Since the application of copper is influenced by the value of the crop being grown (Brown 2008), copper is typically not applied during cover-crops years.

11.2 Container

At one time copper fertilizers were not used to grow pine seedlings in containers. “The fact that they are required in such minute amounts and are natural components of peat, soil, fertilizers, and water makes it improbable that a soil mix will have a deficiency of a minor element” (Baker 1957, p 107). For example, when not fertilized with copper, container-grown pine seedlings growing in peat-vermiculite mix can have 3.9 to 12 µg-Cu g^{-1} in needles (Walker and Huntt 1992; Lambert and Weidensaul 1982). Although the chance of a deficiency using unlimed media is the same as it was in 1957, most container-grown pine seedlings (in the USA) are now fertilized with copper. The amount applied per pine seedling typically varies from 0.02 to 0.15 mg-Cu (Jackson et al. 2012; Dumroese et al. 2013).

Approaches used in container nurseries include: (1) apply copper as part of a slow-release fertilizer; (2) apply copper using foliar applications; (3) using both #1 and #2; (4) apply copper to container walls to prevent root spiraling; (5) rely only on irrigation and media to supply copper (Brix and van den Driessche 1974; Rowan 1977; Donald 1991; Walker and Huntt 1992; Urgiles et al. 2009; Shalizi 2015; Hubbel et al. 2018). In a survey of ten nurseries, seven used method #3 (Starkey et al. 2015).

Even though potting media with 50% peat moss, 40% pearlite and 10% vermiculite may contain only 0.01 µg-Cu g^{-1} (Vashisth et al. 2020), seedlings growing in copper-treated containers do not need to be fertilized with copper (Arnold et al. 1997; Acevedo et al. 2020; Figure 6). In some cases, the coating will increase foliar levels of pine seedlings by more than 1 µg-Cu g^{-1} (Hunt 1990; Rey 1997; South et al. 2005; Tsakaldimi and Ganatsas 2006). The copper coating alters root growth and sometimes results in a significant increase in pine shoot biomass (Aldrete et al. 2002; Sword Sayer et al. 2009). The change in root configuration increases nutrient uptake (Tsakaldimi and Ganatsas 2006) and, therefore, the increase in biomass is not a result of a hidden hunger due to insufficient copper fertilization.

The N:Cu ratio can be determined by examining the fertilizer labels and then converting the N rate to 100. Operational N:Cu ratios for containers may range from 100 to 0.01 (Tinus and McDonald 1979) to 100 to 1.3 (Quoreshi and Khasa 2008). When no lime has been added to the medium, a ratio of 100 to zero can be used (e.g. Lambert and Weidensaul 1982) whenever the irrigation water (3.5 l per seedling) contains at least 0.02 µg-Cu g^{-1} (Landis et al. 1989).

12 Copper products

Fertilizers used in pine seedbeds include CSP ($CuSO_4 \cdot 5H_2O$) and cupric oxide (CuO), which are the most commonly applied copper fertilizers. CSP is often sold as bright blue crystals for controlling algae in ponds while copper (II) oxide appears black. The following is a partial list of copper products that have been used in tree nurseries.

Additional compounds not mentioned below include; cupric chloride, cuprous chloride, cupric nitrate, copper cyanide, copper octanoate, copper ammonium acetate, and copper acetate (Rusjan 2012).

12.1 Copper sulfate

Crystals of CSP contain 25% copper and can be dissolved in warm water and mixed with lime to produce Bordeaux mixture. This fungicide was used in the 19th century and has been applied in organic farms and nurseries to control diseases (Hofmann 1914; Wakeley 1954; Aldhous 1972; Miller and Roach 1980; Keča 2016). "Direct injury by Bordeaux to conifers is practically unknown, and while there has been speculation about possible bad effects from accumulation of copper in the soil through long-continued use, such injury has not been proved in southern pine nurseries" (Wakeley 1954).

Since 1980, CSP has been used as a fertilizer in pine and hardwood nurseries. Rates applied before sowing vary from 8 to 16 kg-CSP ha^{-1} (Aldhous 1972; Davey and McNabb 2019) but some managers applied as much as 50 kg-CSP ha^{-1} (van den Burg 1991). Uniformity is difficult using CSP crystals so many managers apply CSP using sprayers. No injury to bareroot pines was noticed at 67 kg-CSP ha^{-1} (Auten 1945) but ≈120 kg-CSP ha^{-1} injured pine growing in sand in a greenhouse (van Lear and Smith 1972). A rate of 120 kg-CSP ha^{-1} would cost $630, which explains why few managers would apply toxic rates of CSP. For example, three nurseries in Ohio applied a combined total of 34 kg of CSP in 1977 (Miller and Roach 1980). In 2016, over 790,000 kg of CSP were used in California. CSP can be purchased for about $5.25 per kg, which is equivalent to $21 per kg-Cu.

There are different forms of copper sulfate which sometimes results in confusion. For example, one mole of cupric sulfate anhydrous [$CuSO_4$] is 159.6 g, a mole of CSP [$CuSO_4 \cdot 5H_2O$] is 249.7 g, a mole of copper sulfate monohydrate [$CuSO_4 \cdot H_2O$] is 176.9 and a mole of tri-basic copper sulfate [$3Cu(OH)_2 \cdot CuSO_4$] is 416.8 g. Therefore, how many grams does it take to make one mole of copper sulfate (van Stennis 1997)?

12.2 Copper oxychloride

Copper oxychloride ($Cu_2(OH)_3Cl$) is used as a fungicide in pine plantations and nurseries (Ferreira and Muchovei 1990; Dick and Vanner 2008; Keča 2016). When applied to container surfaces to reduce root spiraling, it can affect photosynthesis, transpiration and root-collar diameter; depending upon container type and N rate (Dumroese et al. 2013). It was once the sixth most extensively used substance on all orchard crops in United Kingdom (Mace et al. 2018). Occasionally it has been applied to correct a copper deficiency in nurseries (Knight 1975). Injury can occur by treating pine seed before sowing (Arvidsson 1991) or by treating emerging seedlings seven times or more with high rates of copper (Gibson 1958; Bassett 1960). No injury occurred when treating pine seedlings with a normal rate of 3.4 kg-Cu ha^{-1} (Rolando et al. 2017). When applied in soil before sowing (35 kg a.i. ha^{-1}), this fungicide did not affect ectomycorrhiza of pines (Manninen et al. 1998). In 2016 over 130,000 ha of crops were treated with copper oxychloride in California.

12.3 Copper oxide

Copper oxide comes in two version; red-yellow cuprous oxide (Cu_2O) is a reduced form of the normal, black cupric oxide (CuO). Early trials with yellow cuprous oxide indicated injury to unstratified pine seed, but no injury to stratified seed (Hamilton and Jackson 1951). When used as a fertilizer, black cupric oxide is at least as good as CSP (Reitz and Shimp 1953). In New Zealand, a transition to cuprous oxide (from copper oxychloride) was made for disease control because of price and convenience (Rolando et al. 2016). Tests at two nurseries indicate higher than labeled rates are relatively safe on loblolly pine seedlings (Payne et al. 2019, Table 7). In one Scandinavian trial, pine seedlings treated with copper oxide grew well on a peat soil, perhaps because they were taller at time of planting (Pietilaeinen and Veijalainen 1979). In two trials in New Zealand, injury to foliage was noticed after seedlings treated with cuprous oxide reached near freezing temperatures (Fraser et al. 2019). In 2016 over 52,000 ha of crops in California were treated with copper oxide. Some liquid formulations containing copper oxide sell for about $46 per kg-Cu.

Table 7. Seedling characteristics of loblolly pine (*Pinus taeda* L.) treated with foliar applications of copper (8 weeks after sowing). The product was diluted and applied as a liquid over the top of seedlings (Payne et al. 2019). Copper was derived from copper oxide. Applications were made on June 27 (Nursery A) and July 2 (Nursery B) and seedlings were sampled on November 19, 2018 (Nursery A) and October 16, 2018 (Nursery B). LSD = least significant difference; P = probability of a greater F-value for treatment effects; RCD = root collar diameter.

Nursery	Rate (kg-Cu ha^{-1})	Density ($\#m^2$)	Height (cm)	RCD (mm)	Shoot (g)	Root (g)	Total (g)	Root mass ratio (%)
A	0	308	29.7	4.18	2.61	0.47	3.08	15.2
	1.14	297	29.6	4.15	2.60	0.46	3.06	15.1
	4.58	301	29.6	4.21	2.66	0.46	3.12	14.7
	9.17	315	30.6	4.20	2.57	0.46	3.03	15.2
	LSD=0.1	*36*	*0.9*	*0.26*	*0.33*	*0.05*	*0.37*	*1.04*
	LSD=0.05	*44*	*1.1*	*0.32*	*0.41*	*0.06*	*0.46*	*1.27*
	P values	*0.81*	*0.22*	*0.97*	*0.97*	*0.96*	*0.98*	*0.79*
B	0	242	23.8	3.84	2.40	0.36	2.76	13.2
	1.14	215	24.1	3.87	2.31	0.38	2.69	14.2
	4.58	222	22.5	3.88	2.37	0.37	2.74	13.6
	9.17	224	23.8	4.05	2.43	0.38	2.81	13.5
	LSD=0.1	*25*	*1.5*	*0.32*	*0.39*	*0.08*	*0.46*	*1.01*
	LSD=0.05	*31*	*1.8*	*0.39*	*0.48*	*0.09*	*0.57*	*1.32*
	P values	*0.32*	*0.31*	*0.63*	*0.96*	*0.96*	*0.98*	*0.45*

12.4 Cupric carbonate

Cupric carbonate ($CuCO_3$) was once used to treat pine seedlings in the nursery in hopes it would reduce grazing damage by cattle (Duncan and Whitaker 1959). However, when treated seedlings were packaged together in bales, seedling mortality increased after outplanting (Burns 1960). Since it is phytotoxic to roots, it has been used as a coating to reduce root spiraling in containers (Burdett 1978, Ruehle 1985).

12.5 Copper hydroxide

As a fungicide, this compound ($Cu(OH)_2$) has been used on conifer seedlings in greenhouses and in nurseries (Keča 2016). A rate of 2.5 kg-Cu ha^{-1} did not injure pine seedlings (Pawuk 1983) and 3.4 kg-Cu ha^{-1} was safe on bareroot conifers in Mississippi (Smyly and Filer 1973). Likewise, 1.9 kg-Cu ha^{-1} was safe on outplanted longleaf pine (Kais 1975). When grown in pine bark, baldcypress seedlings grew best when fertilized with 200 g l^{-1} of copper hydroxide (Arnold et al. 1997). Prices vary but one product costs about $70 per kg-Cu. In 2016 over 1,100,000 kg of copper hydroxide was used in California.

12.6 Chelated copper

There are several chelated copper fertilizers on the market. Chelates used for copper include ethylenediaminetetraacetic acid (EDTA), citric acid, fatty acids and lignosulfonate. In theory, chelation makes copper more available for uptake (Davey 2002) but supporting data for pines (with thick epicuticular wax) are lacking (Iyer and Wilde 1974). Labeled rates (for a given crop) vary with lower rates typically recommended for higher priced formulations. For bareroot conifer seedlings, there are no publications showing applying chelated copper results in a positive growth response (Pietilaeinen Veijalainen 1979).

Tolerance to chelated copper depends on species and chelating agent. One product (copper salts of fatty and rosin acids) was applied to pines 12 times (at a rate of 0.18 kg-Cu ha^{-1} per application) with no reported injury to seedlings (Stanosz and Smith 1996). In contrast, a single application of Cu-EDTA (at 21,000 µg g^{-1}) can defoliate some hardwoods (Knight 1983). When applied at 905 l ha^{-1} this is equivalent to 19 kg-Cu-EDTA or 3 kg-Cu ha^{-1}. Due to possible phytotoxicity, some product labels do not recommend applying 1.5 kg of Cu-EDTA ha^{-1} to foliage in greenhouses.

CSP might cost $21 per kg-Cu while chelates may cost $26 to $150 per kg-Cu. Rates prescribed for CSP may be equivalent to 1.1 kg-Cu ha^{-1} while labeled rates for chelated products may be one-fifth that amount. An application of 3 kg-Cu ha^{-1} might cost $63 for CSP or $200 for some Cu-chelate brands.

12.7 Copper naphthenate

This wood treatment ($C_{22}H_{14}CuO_4$) was used to control damping-off in container nurseries (Baker 1957) and was used to treated burlap for use in bareroot nurseries (Anderson and Kinneer 1949; McQuilkin 1950; Kuhns and Sydnor 1976). However, when treated wood was used to make 2.5-cm tall seed flats, seedlings died after emerging roots made contact with copper naphthenate (Ferguson 1960; Wall 1978). In 2016 over 6,500 kg of copper naphthenate was used in California.

12.8 Copper nanoparticles

Copper nanoparticles are engineered structures with dimensions less than 100 nm. Their novelty makes for interesting research but the current retail price (at $560,000 per kg-Cu) limits use to researchers. Copper nanoparticles have been tested on container-grown pine seedlings (Aleksandrowicz-Trzcinska et al. 2018). A total rate of 0.2 kg ha^{-1} (8 applications spread over two years) increased 2-0 Scots pine seedling biomass by 49% (i.e. 2.77 g), even though copper was already supplied by a slow-release fertilizer.

13 Conclusions

A review of the literature results in the following conclusions.

(1) Soil values range from 0.2 to 1.5 µg-Cu g^{-1} (Mehlich 3) in loblolly pine plantations while nurseries in the South range from 0.1 to 6.8 µg-Cu g^{-1}. For pines, there is no need for nursery soil to contain more than 0.6 µg-Cu g^{-1} (Mehlich 3).

(2) When irrigation water contains 0.02 µg-Cu g^{-1} (or more) applying 30 cm of water (over the growing season) should prevent a copper deficiency.

(3) Foliar values of 2 to 3.5 µg-Cu g^{-1} are normal for loblolly pine needles in fascicles. Treating needles with copper fertilizers or fungicides can produce values higher than 20 µg-Cu g^{-1}. The "adequate" range for pine foliage is the same for both container and bareroot stock.

(4) Slash pine with foliar values of 1 µg-Cu g^{-1} and longleaf pine with foliar values of 0.5 µg-Cu g^{-1} did not show visible deficiency symptoms.

(5) Copper residues on foliage can make some assumptions (based on foliar concentrations) incorrect.

(6) For pine seedlings in bareroot nurseries, there is poor correlation between copper availability in the soil and foliar copper content.

(7) It is difficult to prove (statistically) a "hidden hunger" effect for copper since the statistical power of most experiments is low.

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