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Response of Oak and Maple Seed Germination and Seedling Growth to Different Manganese Fertilizers in a Cultured Substratum

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Abstract: Oak regeneration failures have been causing a slow decline in the occurrence of oak forest ecosystems in eastern North America. Accordingly, our study sought to determine a means of creating more vigorous and competitive oak seedlings by the addition of manganese (Mn) fertilizers. Seeds of northern red oak (*Quercus rubra* L.), chestnut oak (*Quercus prinus* L.), and red maple (*Acer rubrum* L.), one of oak's major competitors in North America oak forest ecosystems, were sown in 0.7 liter pots that contained a growing medium mixture of peat moss, perlite, and sand in a ratio of 2:1:2, and germinated in a greenhouse. Three different chemical compound Mn fertilizer treatments—manganese chloride (0.16 mg L^{-1} Mn, $MnCl_2{\cdot}4H_2O$), nanoparticle manganese in the form of manganese hydroxide (0.01 mg/L Mn, nanoparticle $Mn(OH)_2$), and manganese hydroxide (0.01 mg L^{-1} Mn, $Mn(OH)_2$)—and a treatment of Hoagland solution were applied to the planted seed. These treatments were compared to a control consisting of water, and treatments were applied twice a week over a 12 week period. Germination rates and seedling growth were measured over this period of time. At the end of 12 weeks seedlings were harvested, separated into roots, stem, and foliage for the purpose of biomass and nutrient analysis by seedling component. Northern red oak displayed a 100% germination success rate with $MnCl_2{\cdot}4H_2O$ and $Mn(OH)_2$ treatments, while red maple germination was reduced with the $MnCl_2{\cdot}4H_2O$ and nanoparticle $Mn(OH)_2$ treatments with only a 32% and 24% germination rate, respectively. The $MnCl_2{\cdot}4H_2O$ treatment produced the largest overall seedling size (basal diameter squared times the seedling height) of red maple with a 191.6% increase; however, the $MnCl_2{\cdot}4H_2O$ treatment produced the largest overall seedling size (basal diameter squared times the seedling height) of northern red oak and chestnut oak with an increase of 503.7% and 339.5%, respectively. The greatest increase in overall seedling size for northern red oak was with the $Mn(OH)_2$ treatment at 507.2%, and 601.2% for chestnut oak with the nanoparticle $Mn(OH)_2$ treatment. $MnCl_2{\cdot}4H_2O$ treatment significantly increased the oak foliar nitrogen (N) content. It appears that the application of Mn fertilizer can increase the germination and growth of these oak species while suppressing or having a lesser effect on red maple, thus creating a competitive advantage for oak over its competitor.

Keywords: manganese fertilizer; northern red oak; chestnut oak; red maple; seedling growth; seed germination

1. Introduction

Oak (*Quercus* sp.) is an important tree species in forest ecosystems of North America. However, oak forests have been shrinking due to various problems it experiences when regenerating, causing it to lose its dominant status in the Central Hardwoods Region of the eastern United States [1]. The

oak regeneration problem is one of the most important forest management issues today [2,3] and the successful regeneration of oak depends on whether there are enough stems of competitive oak seedlings regenerating on the forest floor before the canopy is disturbed [4]. Oak seedlings typically have low survival during the first year as compared with their shade tolerant red maple competitor. Oak seedlings, for which prescribed fire has been used to help get seedlings established, are fire tolerant while its competitors are not [5–9]. In the absence of burning it is therefore critical that there exist an adequate number of competitive oak seedlings.

Due to the continuous oak regeneration failures that occur in the oak forests of eastern North America, often as a result of low light availability and competition [10,11], there is a need to develop methods that will produce more competitive oak seedlings. Stronger oak seedlings grow faster and store much more nutrients [12,13], allowing them to be more resistant to competition and deficient sunlight [14–17], low site fertility [14,18–20], and herbivore browsing [20,21]. A number of researchers have discovered that applying fertilizer improves the oak seedling quality and growth [22–27]. However, some literature has revealed that fertilizer may have no effect, an ambiguous effect or even a negative effect on oak seedling growth [28–30]. In addition, field application of fertilizer to oak may result in the leaching of applied nutrients away from oak and an inadvertent uptake by its competitors, thus giving competitors an increased advantage [31,32]. A possible way to avoid this problem may be to apply fertilizer that is specific to oak, that is, nutrients or a form of nutrients that oak can use to its advantage that its competitors cannot [33].

There is much evidence to suggest that manganese (Mn) could be one of those nutrients that has a positive effect in oak forests. When ammonium sulfate fertilizer was applied in a temperate hardwood forest located in West Virginia, oak species absorbed more Mn than other species in the same forest [34]. When Mn as a fertilizer was applied in an oak forest located in Ukraine, the Mn content was found to be higher in the oak root layer compared to the control, suggesting that oak has a unique ability to uptake and store Mn [22]. English oak (*Q. robur* L.) and Cornish oak (*Q. petraea* (Matt.) Liebl.) can often grow in forest soils with high soluble manganese (Mn^{2+}) concentrations [35]. The Mn concentration in Faber's oak (*Q. fabri* Hance), a white oak found in the natural secondary forests of China, was 8.68 g hm^{-2} and higher than the content of iron (Fe, 7.18 g hm^{-2}), and much higher than the contents of zinc (Zn, 0.40 g hm^{-2}) and copper (Cu, 0.22 g hm^{-2}) [36]. Some ectomycorrhizal fungal groups associated with oak have been found to be positively correlated with high concentrations of Mn in the soil [37].

Mn is an essential plant microelement that participates in the water-splitting system associated with photosystem II, which provides the electrons for all of photosynthesis to occur. Mn is taken up by plants from the soil solution as Mn^{2+} [38], and Mn^{2+} is responsible for activating many enzymes in the plant [35,39]. When applied to mung bean (*Vigna radiata* (L.) Wilczek) plants, Mn had a positive effect on the root and shoot length, fresh and dry weight, and rootlet number [40]. Mn in the form of nanoparticles has been shown to significantly enhance lettuce (*Lactuca sativa* L.) seed germination and growth [41]. In most plants, the Mn concentration of 0.02 mg g^{-1} in foliar dry mass is sufficient for normal growth, but the concentration necessary will vary by species [35]. Soil conditions, such as soil pH and the concentration of Mn in the soil, will affect the available Mn to plants [42]; but these conditions can be changed by alkaline runoff from surfaces, thereby reducing the solubility of soil manganese. It is this problem that has caused manganese deficiency in white oak (*Q. alba* L.) [43]. Conversely, too much Mn can be toxic to plants when plants uptake excess concentrations of Mn^{2+}, resulting in growth reduction and impairment of chlorophyll synthesis [44].

Therefore, the purpose of this study was to determine effects of Mn fertilizer applied in different compositions on the germination, seedling morphology, and mineral nutrition of northern red oak (*Q. rubra* L.), chestnut oak (*Q. prinus* L.), and red maple (*Acer rubrum* L.). Northern red oak and chestnut oak are two important species widely distributed in eastern North America forest ecosystems and red maple is one of their major competitors. We hypothesized that oak seedlings will exhibit increased growth and biomass with the Mn chemical compound application. Different Mn compounds

or morphology were used in this study including manganese chloride ($MnCl_2$), manganese hydroxide ($Mn(OH)_2$), and Mn nanoparticle ($Mn(OH)_2$). Specifically, we wanted to find out the effects of Mn chemical compounds added treatments on the germination and seedling morphology and mineral nutrition of oak and maple, and if any particular Mn compound provides a better outcome.

2. Materials and Methods

2.1. Plant Material and Experiment Conditions

All seeds used in this experiment were dormant seeds purchased from a seed supply company located in the Midwestern United States. Seeds were washed and soaked in tap water for 24 h in the laboratory at a temperature of 20 ± 2 °C. The floating test was applied to all seeds to determine potential viability. Seeds that floated and displayed signs of diseases and pests were discarded. The remaining seeds were stratified by storing them in moist polyethylene bags at 3–5 °C for two months to assure the breaking of dormancy.

Each species' (northern red oak, chestnut oak, red maple) seeds were sown in 3.8 liter pots that contained a growing medium mixture of peat moss, perlite and sand in a ratio of 2:1:2. Seeds were sown on 6 August 2015, to a depth of 3 cm for oak seeds and 2 cm for red maple seeds. Only natural sunlight was utilized in the greenhouse, and the temperature was maintained at 25 °C during the day and 22 °C at night. Seedling treatments were arranged in a randomized block design.

Seeds and seedlings were cultured under one of four different treatments: (1) Manganese in the form of manganese chloride (0.16 mg L^{-1} Mn, $MnCl_2 \cdot 4H_2O$) dissolved in Hoagland solution, referred to hereafter as TMn; (2) nanoparticle manganese in the form of manganese hydroxide (0.01 mg L^{-1} Mn, nanoparticle $Mn(OH)_2$) [41] dissolved in Hoagland solution, referred to hereafter as NMn; (3) manganese hydroxide (0.01 mg L^{-1} Mn, $Mn(OH)_2$) in suspension solution dissolved in Hoagland solution, referred to hereafter as SMn; and (4) Hoagland solution comprised with macronutrients such as N (210 mg L^{-1}), P (31 mg L^{-1}), K (235 mg L^{-1}), and micronutrients such as Mn (0.5 mg L^{-1}) referred to hereafter as HS. The control (C) was normal tap water from the greenhouse. Nutrient solutions were applied over a 12-week period, with 25 mL of treatment solution being applied to each pot twice a week. The control, which consisted of 50 mL of tap water, was applied to each pot once a week.

2.2. Morphological Characteristics

Seeds began germination five days after being sown. The initiation of germination was recorded when the bud first broke the surface of the substratum, without distinguishing the epicotyls for oak or hypocotyls for red maple. The number of germinated seeds was recorded each day by species and treatment until the number of seedlings emerging decreased substantially. Seedling height (nearest 0.01 cm), basal diameter (nearest 0.01 mm) and number of leaves were measured and recorded every two weeks. Twelve weeks after the seeds were sown, the whole seedling was harvested and carefully washed with tap water to remove soil medium from the root system. Ten seedlings of each species (northern red oak, chestnut oak, red maple) were randomly selected for each treatment and control, with each treatment and control replicated. This provided a total of 30 seedlings samplings of each species in each treatment and control.

Each seedling was separated into leaves, stem and roots to obtain the fresh weight of each seedling component to the nearest 0.01 g. However, the red maple seedlings were much smaller than the northern red and chestnut oaks and not enough plant material were able to be collected to analyze by component. Therefore, the entire red maple plant was used rather than separating it into components. Each seedling component was oven dried for 72 h at 60 °C to determine the moisture content and subsequent dry weight.

2.3. Plant Element Concentration

Twelve seedlings of each species were randomly selected from each treatment for the purpose of nutritional analysis of each seedling component. These samples were ground in a Wiley mill to pass a 2 mm sieve and used to determine nutrient content. Total nitrogen was determined by the Kjeldahl method [45], and the remaining elements were determined by ICP analysis after digestion, ashing the samples at 500 °C and dissolving them in 10% nitric acid solution [46].

2.4. Data Analysis

A one-way ANOVA was used to analyze the effect of fertilization treatment on germination, morphological characteristics and nutritional content. Differences between means were assessed by Duncan's multiple range tests using $p \leq 0.05$. All analyses were conducted in SPSS (IBM ver. 20.0).

3. Results

3.1. Seed Germination

Among the species, northern red oak displayed the highest germination success, measured as percent of seeds germinated, across all treatments with an average of 95% compared to an average of 69% for chestnut oak and 62% for red maple (Table 1). Most of the northern red oak germinated during the second week after being sown whereas chestnut oak germinated mainly during the third and the fourth week.

Table 1. Mean [1] seed germination success based on the percentage of seeds that germinated among northern red oak (*Q. rubra* L.), chestnut oak (*Q. prinus* L.), and red maple (*Acer rubrum* L.) with different treatments [2].

Treatment	Species Germination (%) ± SE		
	Northern Red Oak	Chestnut Oak	Red Maple
C	75.7 ± 0.3a	71.7 ± 0.3c	72.0 ± 0.6c
HS	96.0 ± 0.6c	84.3 ± 0.3e	79.0 ± 0.6d
TMn	100.0 ± 0.0d	80.3 ± 0.3d	57.0 ± 0.6b
NMn	84.7 ± 1.2b	54.7 ± 0.9a	41.0 ± 0.9a
SMn	100.0 ± 0.0d	58.3 ± 0.7b	71.0 ± 0.7c

[1] Different letters indicate significant differences between treatments within each species at $p = 0.05$, Duncan's MRT. [2] C = control; HS = Hoagland solution; TMn = manganese ($MnCl_2 \cdot 4H_2O$) + Hoagland solution; NMn = nanoparticle manganese ($Mn(OH)_2$) + Hoagland solution; and SMn = suspension manganese ($Mn(OH)_2$) + Hoagland solution.

All treatments produced significantly higher germination success rates (84.7%–100.0%) compared to the control (75.7%) for northern red oak. TMn and SMn treatments produced 100% germination success for northern red oak. The HS treatment produced the second highest success at 96%. The NMn treatment produced the lowest germination success rate of all treatments (84.7%), but was still significantly higher than the control.

Only the TMn and HS treatments produced significantly higher germination success compared to the control (71.7%) for chestnut oak at 80.3% and 84.3%, respectively. However, both the SMn and NMn treatments seemed to have a suppressing effect on chestnut oak germination as the germination success was significantly lower at 58.3% and 54.7%, respectively

Only the HS treatment produced higher germination success (79.0%) compared to the control (72.0%) for red maple. All Mn treatments resulted in lower germination success, with the TMn and NMn treatments producing significantly lower success at 57.0% and 41.0%, respectively. The SMn treatment produced lower but non-significant germination success compared to the control at 71.0%.

In summary, these results revealed that Mn treatments improved northern red oak seed germination, as northern red oak had a 100% germination success with the TMn and SMn treatments. The application of Mn appeared to have no or a suppressing effect on red maple germination. However,

there was a mixed effect on chestnut oak germination as the TMn treatment improved germination while the NMn and SMn treatments appeared to have a suppressing effect.

3.2. Morphology and Biomass of Seedlings

All fertilization treatments had a significant effect ($p < 0.05$) on seedling height, diameter, and leaf number of northern red oak, and a significant effect on chestnut oak, and red maple seedling height when compared to the control (Table 2). The greatest seedling height growth of northern red oak was 24.89 ± 0.69 cm (mean ± SE) with the NMn treatment; for chestnut oak was 18.10 ± 1.28 cm with the SMn fertilizer treatment; and for red maple was 8.9 ± 0.12 cm with the TMn treatment.

Table 2. Mean [1] of total height, basal diameter, and number of leaves of 12-week-old seedlings by species following treatments [2] of fertilizer with and without manganese.

Species	Treatments	Mean Seedling Attribute ± SE		
		Height (cm)	Diameter (mm)	Leaf Number
Northern red oak	C	9.65 ± 0.46a	3.71 ± 0.31a	6.8 ± 0.9a
	HS	21.16 ± 0.88b	5.42 ± 0.24b	13.6 ± 1.5b
	TMn	24.24 ± 0.58c	5.75 ± 0.20b	12.2 ± 1.1b
	NMn	24.89 ± 0.69c	5.58 ± 0.31b	12.2 ± 0.4b
	SMn	24.45 ± 0.72c	5.74 ± 0.21b	11.6 ± 0.3b
Chestnut oak	C	6.53 ± 0.12a	2.68 ± 0.23a	4.9 ± 0.3a
	HS	10.03 ± 0.39b	2.87 ± 0.28a	7.0 ± 0.7ab
	TMn	15.63 ± 0.59c	3.64 ± 0.32ab	8.8 ± 0.6bc
	NMn	17.20 ± 1.72c	4.38 ± 0.49b	12.7 ± 0.9d
	SMn	18.10 ± 1.28c	3.93 ± 0.36b	11.5 ± 1.7cd
Red maple	C	5.53 ± 0.18a	2.86 ± 0.15a	6.3 ± 0.3a
	HS	7.18 ± 0.42b	3.15 ± 0.05ab	8.8 ± 0.9ab
	TMn	8.90 ± 0.12c	3.85 ± 0.38c	13.0 ± 1.3c
	NMn	7.93 ± 0.61bc	3.21 ± 0.08ab	11.8 ± 2.0bc
	SMn	8.70 ± 0.21c	3.50 ± 0.35ab	12.3 ± 0.9bc

[1] Different letters indicate significant differences between treatments within each species at $p = 0.05$, Duncan's MRT. [2] C = control; HS = Hoagland solution; TMn = manganese ($MnCl_2 \cdot 4H_2O$) + Hoagland solution; NMn = nanoparticle manganese ($Mn(OH)_2$) + Hoagland solution; and SMn = suspension manganese ($Mn(OH)_2$) + Hoagland solution.

When compared to the control, all Mn treatments significantly increased the height growth of northern red oak from 151% to 158%, whereas the HS treatment increased height growth significantly by 119%. For chestnut oak, all Mn treatments likewise increased height growth significantly from 139% to 177%. While the HS treatment significantly increased the height growth of chestnut oak, it increased by only 54%. All treatments had a significant but less of an effect on red maple compared to the oaks, where the Mn treatments increased red maple seedling height growth from 43% to 60%, while the HS treatment increased the height growth by only 30%.

All treatments significantly increased the basal diameter growth of northern red oak compared to the control, with no significant differences among treatments (Table 2). There was an average increase among all treatments of 51.5% with the TMn treatment producing the greatest increase of 55.0%. For chestnut oak only the NMn and SMn treatments significantly increased basal diameter growth compared to the control, producing increases of 63.4% and 46.6%, respectively. Non-significant increases of 7.1% and 35.8% were produced by the HS and TMn treatments, respectively.

All treatments had less of an impact on red maple basal diameter growth compared to the oak species with an overall average increase of 19.8% (Table 2). Only the TMn significantly increased the basal diameter growth with an increase of 34.6%. Although not significant, the NMn and SMn treatments caused a greater increase in basal diameter growth then the HS treatment.

If we quantify the overall seedling size as the basal diameter squared times the seedling height, we found that the treatments, particularly the Mn treatments, increased the overall oak seedling size to

a greater extent than red maple. Even though the red maple seedlings were inherently smaller, greater gains were made by the oak seedlings (Figure 1). The Mn treatments increased the overall seedling size more than the HS treatment. The TMn treatment produced the greatest increase in size of red maple with a 191.6% increase, but this treatment increased the overall size of northern red oak and chestnut oak by 503.7% and 339.5%, respectively. The greatest increase in overall seedling size for northern red oak occurred with the SMn treatment at 507.2%, and for chestnut oak occurred with the NMn treatment at 601.2%.

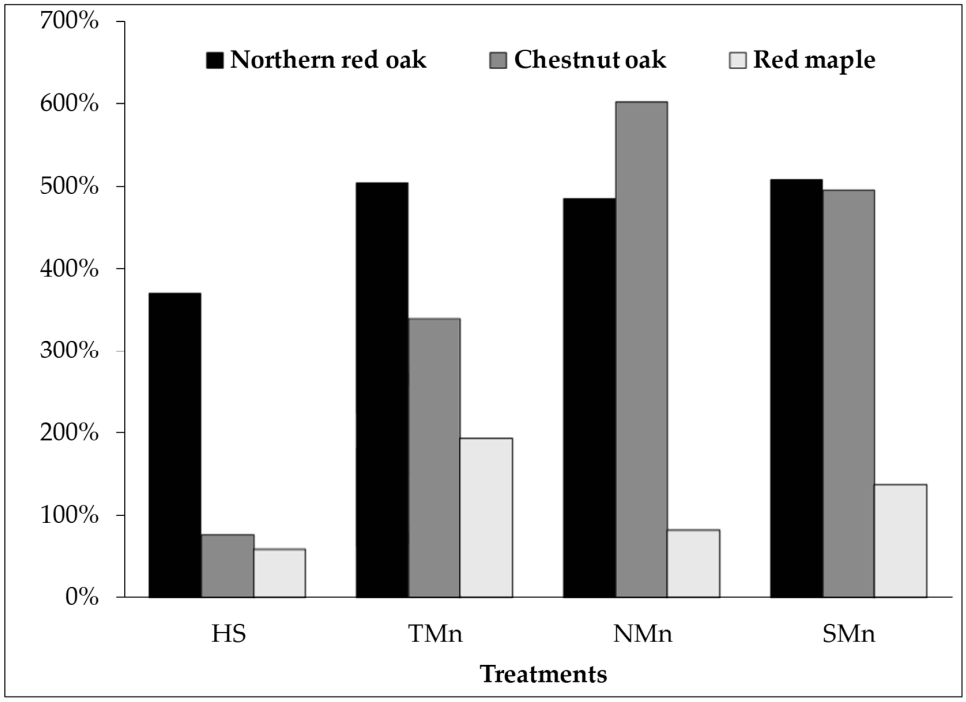

Figure 1. Percent changes based on the mean overall seedling size (basal diameter squared × total height) compared to the control for each treatment by species. The treatments are: HS = Hoagland solution; TMn = manganese ($MnCl_2 \cdot 4H_2O$) + Hoagland solution; NMn = nanoparticle manganese ($Mn(OH)_2$) + Hoagland solution; and SMn = suspension manganese ($Mn(OH)_2$) + Hoagland solution.

Treatments used in this study had a significant effect on northern red oak biomass, except for the stem and leaf biomass (Table 3). The TMn treatment produced the greatest biomass increase for northern red oak whether you consider the whole plant or root, which were 12.09 ± 0.02 g and 5.52 ± 0.30 g, respectively.

Similarly, treatments caused significant increase in the whole seedling biomass and the biomass of the seedling components for chestnut oak. The greatest biomass increase occurred with the NMn treatment for the whole plant (4.15 ± 0.56 g), root (1.17 ± 0.24 g), stem (1.09 ± 0.18 g), and foliage (1.89 ± 0.16 g).

The overall response of the red maple seedling biomass was of a lesser magnitude than that of the oaks, with only the foliage biomass displaying a significant increase. The TMn treatment produced the greatest foliar biomass increase (0.32 ± 0.03 g) as well as the greatest total seedling biomass increase (0.61 ± 0.02 g).

Compared to the control, the TMn treatment produced the largest increase in total and root biomass for northern red oak of 123.1% and 110%, respectively. The NMn treatment caused the largest stem increase for northern red oak of 151.2% while the HS treatment resulted in the largest foliage increase at 139.6%. For chestnut oak, the NMn treatment caused the largest increase for root, stem, and foliage with increases of 67.1%, 303.7%, and 397.4%, respectively. TMn treatment caused the largest increases in total, stem, and foliage for red maple at 369.2%, 300.0%, and 966.7%, respectively. The

SMn treatment caused a comparable increase of stem biomass for red maple as the TMn treatment, and the SMn treatment caused the largest root biomass increase of 183.3%. Except for the foliage biomass of northern red oak, the Mn treatments produced the greatest biomass gains in the different seedling components as well as the total biomass.

Table 3. Mean [1] dry biomass (g) of 12-week-old seedlings by species and component following treatments [2] of fertilizer with and without manganese.

Species	Treatments	Mean Biomass (g) ± SE			
		Total	Root	Stem	Leaf
Red oak	C	5.42 ± 0.26a	2.62 ± 0.19a	1.27 ± 0.24a	1.54 ± 0.11a
	HS	10.34 ± 0.20b	3.82 ± 0.09b	2.83 ± 0.21b	3.69 ± 0.14b
	TMn	12.09 ± 0.02c	5.52 ± 0.30c	3.09 ± 0.14b	3.47 ± 0.18b
	NMn	10.11 ± 0.58b	3.25 ± 0.62ab	3.19 ± 0.14b	3.67 ± 0.10b
	SMn	10.10 ± 0.26b	3.46 ± 0.04ab	3.09 ± 0.32b	3.55 ± 0.46b
Chestnut oak	C	1.35 ± 0.08a	0.70 ± 0.08ab	0.27 ± 0.03a	0.38 ± 0.03a
	HS	1.56 ± 0.08ab	0.62 ± 0.03a	0.35 ± 0.05a	0.63 ± 0.06b
	TMn	2.18 ± 0.21b	0.58 ± 0.17a	0.52 ± 0.05a	1.04 ± 0.03c
	NMn	4.15 ± 0.56d	1.17 ± 0.24b	1.09 ± 0.18c	1.89 ± 0.16e
	SMn	3.36 ± 0.16c	1.06 ± 0.03ab	0.81 ± 0.13b	1.50 ± 0.00d
Red maple	C	0.13 ± 0.02a	0.06 ± 0.01a	0.03 ± 0.01a	0.03 ± 0.00a
	HS	0.32 ± 0.07b	0.10 ± 0.03ab	0.08 ± 0.02b	0.14 ± 0.03a
	TMn	0.61 ± 0.02c	0.17 ± 0.05b	0.12 ± 0.01b	0.32 ± 0.03b
	NMn	0.56 ± 0.08c	0.16 ± 0.01b	0.11 ± 0.01b	0.28 ± 0.08b
	SMn	0.59 ± 0.05c	0.17 ± 0.03b	0.12 ± 0.01b	0.30 ± 0.02b

[1] Different letters indicate significant differences between treatments within each species at $p = 0.05$, Duncan's MRT. [2] C = control; HS = Hoagland solution; TMn = manganese ($MnCl_2 \cdot 4H_2O$) + Hoagland solution; NMn = nanoparticle manganese ($Mn(OH)_2$) + Hoagland solution; and SMn = suspension manganese ($Mn(OH)_2$) + Hoagland solution.

The Mn fertilizer improved the oak and maple biomass compared with the control and the N, P, and K additions with the HS treatment. The HS treatment increased the total seedling biomass for northern red oak, chestnut oak, and red maple by 4.92 g, 0.21 g, and 0.19 g, respectively, compared to the control. However, the average increases in total biomass for the Mn treatments were 5.35 g, 1.88 g, and 0.46 g for northern red oak, chestnut oak, and red maple, respectively, compared to the control. These treatments caused a redistribution of biomass among the seedling components. The root system and foliage biomass comprised 52% and 28%, respectively, for chestnut oak in the control. The HS treatment changed this distribution to 40% and 40% for the root system and foliage, respectively. The Mn treatments caused this distribution to become more heavily to foliage as the average distribution percentage was 29% and 46% for the root system and foliage, respectively.

A similar trend was observed for northern red oak as the distribution of dry biomass for the root system and foliage in the control was 48% and 28%, respectively. The HS treatment changed the distribution of root and foliage biomass to 37% and 36%, respectively. The average redistribution of the root and foliage biomass in the Mn treatments was 37% and 45%, respectively.

3.3. Plant Element Concentrations

Northern red oak accumulated higher concentrations of elements in the foliage compared to the stem and root (Table 4). The elements N and P were significantly higher in all seedling components across all treatments when compared to the control. The elements K and Fe in the roots were significantly higher than the control in the Mn treatments and SMn treatments, respectively. However, there were no significant differences of Mg concentrations in all the northern red oak seedling components, and no significant differences of K (except SMn treatment in stem) and Mn concentrations in foliage and stem components across all treatments.

Table 4. Effect of manganese fertilization treatments [1] (Treat) on the mean [2] elemental content of 12-week-old northern red oak (*Quercus rubra* L.) seedlings by component (Comp).

Comp.	Treat.	Mean Element Concentration ± SE					
		N	P	K	Mg	Fe	Mn
		(%)	(mg g^{-1})	(mg g^{-1})	(mg g^{-1})	(mg g^{-1})	(mg g^{-1})
Foliage	C	1.27 ± 0.15a	0.70 ± 0.05a	9.34 ± 0.08a	4.10 ± 0.31a	0.05 ± 0.01a	0.51 ± 0.06a
	HS	1.76 ± 0.06b	1.15 ± 0.03b	9.33 ± 0.36a	3.66 ± 0.07a	0.07 ± 0.00a	0.38 ± 0.06a
	TMn	2.36 ± 0.02c	1.27 ± 0.07bc	9.67 ± 0.50a	3.91 ± 0.27a	0.07 ± 0.00a	0.37 ± 0.06a
	NMn	2.15 ± 0.14bc	1.38 ± 0.07bc	10.10 ± 0.91a	3.91 ± 0.30a	0.07 ± 0.01a	0.44 ± 0.08a
	SMn	2.14 ± 0.02bc	1.41 ± 0.11c	9.62 ± 0.26a	4.04 ± 0.26a	0.07 ± 0.01a	0.50 ± 0.08a
Stem	C	0.40 ± 0.05a	0.72 ± 0.04a	3.83 ± 0.13a	1.73 ± 0.22a	0.02 ± 0.00a	0.28 ± 0.02a
	HS	0.63 ± 0.02ab	1.41 ± 0.02ab	4.53 ± 0.54ab	2.03 ± 0.08a	0.02 ± 0.00ab	0.16 ± 0.03a
	TMn	0.81 ± 0.05b	1.48 ± 0.13b	3.95 ± 0.20ab	2.20 ± 0.19a	0.04 ± 0.00b	0.17 ± 0.02a
	NMn	0.76 ± 0.07b	1.43 ± 0.22ab	4.09 ± 0.14ab	2.13 ± 0.27a	0.03 ± 0.00ab	0.25 ± 0.05a
	SMn	0.80 ± 0.09b	1.64 ± 0.22b	4.87 ± 0.51b	2.16 ± 0.06a	0.04 ± 0.01b	0.22 ± 0.06a
Root	C	0.63 ± 0.03a	0.81 ± 0.04a	4.71 ± 0.33a	1.59 ± 0.21a	0.05 ± 0.01ab	0.09 ± 0.00c
	HS	0.84 ± 0.06ab	1.40 ± 0.06ab	6.70 ± 0.53ab	1.48 ± 0.03a	0.04 ± 0.00a	0.02 ± 0.00ab
	TMn	1.18 ± 0.10c	1.73 ± 0.30b	7.79 ± 0.30b	1.93 ± 0.13a	0.06 ± 0.00ab	0.03 ± 0.01ab
	NMn	1.02 ± 0.11bc	1.39 ± 0.09ab	7.13 ± 1.06a	1.65 ± 0.12a	0.05 ± 0.01ab	0.04 ± 0.01b
	SMn	1.13 ± 0.12bc	1.74 ± 0.26b	7.01 ± 0.09ab	1.80 ± 0.15a	0.07 ± 0.00c	0.02 ± 0.00a

[1] C = control; HS = Hoagland solution; TMn = manganese ($MnCl_2 \cdot 4H_2O$) + Hoagland solution; NMn = nanoparticle manganese ($Mn(OH)_2$) + Hoagland solution; and SMn = suspension manganese ($Mn(OH)_2$) + Hoagland solution. [2] Different letters indicate significant differences between treatments within each species at $p = 0.05$, Duncan's MRT.

The TMn treatment produced the highest concentration of N in northern red oak foliage (2.36 ± 0.02 mg g^{-1}) and the highest concentration of N and K in the root component (1.18 ± 0.10 mg g^{-1} and 7.79 ± 0.30 mg g^{-1}, respectively). The SMn treatment produced the highest concentration of Fe (0.07 ± 0.00 mg g^{-1}) in the root component.

The highest concentrations of N, K, Mg, and Mn accumulated in the foliage of chestnut oak while the highest concentrations of P and Fe accumulated in the roots (Table 5). The concentrations of P in the chestnut oak foliage, stem, and root components were significantly different among all treatments compared to the control. The concentrations of N, K, Fe, and Mn in the root component were likewise significantly different among all treatments. The HS treatment produced the highest concentrations of N (2.68 ± 0.18 mg g^{-1}) in the foliage. The highest concentrations of K (16.35 ± 1.94 mg g^{-1}) and Fe (0.12 ± 0.01 mg g^{-1}) were found in the root component of the SMn and NMn treatments, respectively.

Table 5. Effect of manganese fertilization treatments [1] (Treat) on the mean [2] elemental content of 12-week-old chestnut oak (*Quercus prinus* L.) seedlings by component (Comp).

Comp	Treat	Mean Element Concentration ± SE					
		N	P	K	Mg	Fe	Mn
		(%)	(mg g^{-1})	(mg g^{-1})	(mg g^{-1})	(mg g^{-1})	(mg g^{-1})
Foliage	C	2.17 ± 1.13a	1.38 ± 0.14a	10.22 ± 0.03a	3.08 ± 0.07ab	0.05 ± 0.00a	0.55 ± 0.10a
	HS	2.68 ± 0.18a	3.13 ± 0.09c	10.60 ± 0.13a	2.56 ± 0.12a	0.05 ± 0.00a	0.41 ± 0.02a
	TMn	2.52 ± 0.07a	2.66 ± 0.31bc	11.64 ± 0.15a	2.88 ± 0.31ab	0.05 ± 0.00a	0.51 ± 0.10a
	NMn	2.38 ± 0.04a	2.17 ± 0.15b	12.34 ± 0.30a	3.33 ± 0.28c	0.06 ± 0.00a	0.66 ± 0.09a
	SMn	2.33 ± 0.02a	2.33 ± 0.09b	11.75 ± 1.54a	3.50 ± 0.20c	0.05 ± 0.00a	0.48 ± 0.10a
Stem	C	1.14 ± 0.36a	1.70 ± 0.10a	7.69 ± 0.03a	1.80 ± 0.06a	0.04 ± 0.00a	0.63 ± 0.07a
	HS	1.45 ± 0.11a	3.43 ± 0.14b	9.17 ± 0.34a	2.41 ± 0.22b	0.04 ± 0.00a	0.64 ± 0.08a
	TMn	1.45 ± 0.06a	3.62 ± 0.08b	11.51 ± 0.96a	2.38 ± 0.14b	0.03 ± 0.00a	0.58 ± 0.03a
	NMn	1.59 ± 0.22a	3.40 ± 0.26b	9.61 ± 0.90a	2.55 ± 0.08b	0.05 ± 0.01a	0.64 ± 0.09a
	SMn	1.53 ± 0.30a	3.48 ± 0.15b	8.94 ± 1.06a	2.51 ± 0.11b	0.05 ± 0.01a	0.49 ± 0.06a

Table 5. *Cont.*

Comp	Treat	Mean Element Concentration ± SE					
		N	P	K	Mg	Fe	Mn
		(%)	(mg g^{-1})	(mg g^{-1})	(mg g^{-1})	(mg g^{-1})	(mg g^{-1})
Root	C	1.41 ± 0.21a	2.20 ± 0.12a	6.75 ± 0.03a	1.73 ± 0.07a	0.07 ± 0.00a	0.16 ± 0.00c
	HS	2.95 ± 0.00c	4.43 ± 0.04b	9.35 ± 0.04a	1.92 ± 0.00a	0.08 ± 0.01a	0.11 ± 0.00b
	TMn	2.91 ± 0.01c	4.31 ± 0.04b	12.92 ± 1.22b	1.97 ± 0.20a	0.08 ± 0.00a	0.09 ± 0.01ab
	NMn	2.80 ± 0.39bc	3.35 ± 0.26b	9.70 ± 0.65ab	1.95 ± 0.21a	0.12 ± 0.01b	0.09 ± 0.01ab
	SMn	1.99 ± 0.37ab	3.31 ± 0.14ab	16.35 ± 1.94c	1.93 ± 0.09a	0.05 ± 0.01a	0.08 ± 0.01a

[1] C = control; HS = Hoagland solution; TMn = manganese ($MnCl_2 \cdot 4H_2O$) + Hoagland solution; NMn = nanoparticle manganese ($Mn(OH)_2$) + Hoagland solution; and SMn = suspension manganese ($Mn(OH)_2$) + Hoagland solution. [2] Different letters indicate significant differences between treatments within each species at $p = 0.05$, Duncan's MRT.

The concentrations of N, P, K, and Mg, in the red maple seedling were significantly higher in all treatments compared to the control (Table 6). The concentrations of Fe were significantly higher than the control in only the Mn treatments, but the concentrations of Mn were not significantly different from the control in all treatments.

Table 6. Effect of manganese fertilization treatments [1] (Treat) on the mean [2] elemental content of 12-week-old red maple (*Acer rubrum* L.) seedlings.

Treat	Mean Element Concentration ± SE					
	N	P	K	Mg	Fe	Mn
	(%)	(mg g^{-1})	(mg g^{-1})	(mg g^{-1})	(mg g^{-1})	(mg g^{-1})
C	1.50 ± 0.20a	1.55 ± 0.05a	9.18 ± 0.40a	2.33 ± 0.33a	0.06 ± 0.01a	0.88 ± 0.04a
HS	3.67 ± 0.127bc	5.83 ± 0.21b	17.84 ± 0.57b	4.12 ± 0.08b	0.07 ± 0.00ab	0.67 ± 0.04a
TMn	3.53 ± 0.19b	6.65 ± 0.73bc	23.16 ± 1.43c	4.27 ± 0.04b	0.08 ± 0.00b	0.82 ± 0.07a
NMn	4.14 ± 0.18c	8.47 ± 0.52d	24.17 ± 1.19c	4.79 ± 0.63b	0.09 ± 0.01b	0.62 ± 0.31a
SMn	3.48 ± 0.07b	7.64 ± 0.33cd	23.04 ± 0.21c	4.84 ± 0.14b	0.09 ± 0.01b	0.76 ± 0.13a

[1] C = control; HS = Hoagland solution; TMn = manganese ($MnCl_2 \cdot 4H_2O$) + Hoagland solution; NMn = nanoparticle manganese ($Mn(OH)_2$) + Hoagland solution; and SMn = suspension manganese ($Mn(OH)_2$) + Hoagland solution. [2] Different letters indicate significant differences between treatments within each species at $p = 0.05$, Duncan's MRT.

The HS treatment resulted in the highest concentrations of N and P in chestnut oak compared to other treatments, and the highest accumulation was in the root system for both elements (42% and 40%, respectively). The TMn and SMn treatments produced the highest concentration of total N and P, respectively, in northern red oak. The highest accumulation of N from the TMn treatment occurred in the foliage (54%) while the highest accumulation of P from the SMn treatment occurred in the root system (36%). The SMn treatment produced the highest total concentrations of K for both oak species, with the highest accumulation in the roots (44%) for chestnut oak and in the foliage (45%) for northern red oak.

The northern red oak had the greatest foliage N concentration (2.36%), and the greatest total N concentration (4.35%) with the TMn treatment. The TMn treatment also produced the largest root biomass (46% of the total biomass) of all the treatments. However, the HS treatment produced the highest foliar N concentration (2.68%) and total N concentration (7.08%) for chestnut oak, but the lowest root biomass, even lower than the control. The NMn treatment, which produced the greatest amount of root biomass (47%) more than the HS treatment) for chestnut oak, had only 11% less foliar N than the HS treatment.

4. Discussion

Because acorns have an abundance of nutrients, starches, and proteins stored in the endosperm for germination and early seedling development, exogenous nutrients such as N may have no effect on

acorn germination. For example, Holm oak acorns received little germination benefit by exogenous N (19.6 mg L^{-1}), but benefitted only after the second shoot flush [18]. While it has been demonstrated that application of Mn can improve the germination of plants [47], the effects will be determined by the concentration, form, or oxidation state of Mn [48] and the particular plant species. Adding chemical compounds of Mn treatments have the potential to improve the germination of northern red oak and chestnut oak, but suppress the germination of red maple. In a forest setting this could give oak an advantage in seedling establishment and subsequently a competitive advantage over red maple [49–51].

N, P, and K, along with macro and micro elements nutrients, are known to benefit oak seedling growth. Research has demonstrated that holm oak seedlings depended greatly upon N stored in the acorn until about 3 months after seed was sowed [18]. N fertilizers have also been demonstrated to improve the seedling height and stem diameter growth of 24-month-old northern red oak seedlings [52], 3-month-old holm oak height and diameter [26], and cork oak (*Quercus suber* L.) seedling growth [25]. While we found in our study that the HS treatment, which contained high amounts of N, significantly improved the seedling height growth of 3-month-old northern red oak seedlings, the Mn treatments increased the height growth significantly more. Thus it is possible that northern red oak can benefit to a greater extent with Mn than with N fertilizers.

Holm oak acquired a shoot dry biomass of 2.27 g with 60 mg of N, P, and K added for 10 months [26]. In our study, chestnut oak, which also is a white oak as holm oak, had a shoot dry biomass of only 0.63 g after 3 months with the HS treatment; however, the NMn treatment produced a shoot dry biomass of 1.89g when cultured for 3 months. This is just less than half the mass in about one-third the time compared to the study of holm oak with a similar HS treatment [26].

In our study, the highest P concentration was 6.28 mg g^{-1} in the combined stem and foliage fraction of chestnut oak with the TMn treatment (126 mg N and 31 mg P for 3 months). As a comparison with holm oak (*Quercus ilex* L.) within the same fraction, it was discovered that the P element concentration was 1.1 mg g^{-1} (56 mg N and 27 mg P) for 6 months' growth [23] and 2.91 mg g^{-1} P for 7 months growth [26].

Oak has a different growth strategy soon after germination compared to red maple, as oak focuses on root development instead of shoot growth, while red maple is the opposite [53,54]. This results in oak having larger roots than red maple of comparable size [55]. While the treatments in this study redistributed the biomass to the upper portions of the seedling, in particular the foliage, it is unclear what the long-term effect would be in an outplanting situation. The more rapid height growth and increased foliage area should enable these relatively shade-intolerant species to become more competitive at least initially.

The improved biomass growth of seedlings from the Mn treatments can improve the seedling's resistance to the negative effects on early growth from soil compaction due to logging operations [56] and can improve the seedling's competitive advantage. Apart from site quality and amount of available sunlight, one of the critical factors of oak regeneration success in a forest setting is the abundance and size of oak seedlings, where larger oak seedlings enable them to be more competitive [49–51].

The use of fertilizers containing Mn increases the efficiency of photosynthesis and carbohydrate synthesis in plants, thereby increasing their growth and yield [57]. Although Mn is essential for the growth and survival of plants by increasing photosynthetic efficiency, it also can become toxic [48,57,58]. Mn concentrations of approximately 0.02 mg g^{-1} dry mass in foliage is sufficient for normal plant growth, but it can vary by a factor of 60 or more among species [44]. Accordingly, critical concentration thresholds for Mn toxicity vary widely among trees [35]. The critical foliar dry mass Mn concentrations of four Australian tree species ranged from 0.27 mg g^{-1} for candelabra wattle (*Acacia holosericea* A.Cunn. ex G.Don) to 7.23 mg g^{-1} for river red gum (*Eucalyptus camaldulensis* Dehnh.) [58].

None of the seedlings in our experiment displayed signs of Mn toxicity, either through visible signs in the foliage, reduced plant growth, or lowered foliar concentrations of Fe and Mg [39,59]. It is only when metals, such as Mn, are present in bioavailable forms and at excessive levels that they

have the potential to become toxic to plants [60,61]. The NMn treatment produced the highest foliar concentration of Mn of 0.66 mg g^{-1} compared to the Mn concentration of 0.55 mg g^{-1} in the control for chestnut oak. This treatment also produced the highest basal diameter growth and number of leaves per seedling, and the second highest height growth. The analysis of northern red oak found that the control contained the highest concentration of Mn at 0.51 mg g^{-1}, and only very slightly more than the SMn treatment, which was 0.50 mg g^{-1}. The SMn treatment produced the tallest northern red oak seedlings of all treatments and was significantly taller than the control.

5. Conclusions

The results of our experiment demonstrated that oak germination can respond positively to Mn-added nutrient solutions, and suppress the germination of red maple. The germination of northern red oak was improved with Mn treatments when compared to the control and HS treatment. The germination of chestnut oak was improved compared to the control with only the TMn treatment. While the HS treatment improved the germination of red maple over the control, all the Mn treatments suppressed red maple germination. The Mn chemical compound increased the overall seedling size of all three species compared to the HS treatment and control, with the greatest increase observed with the two oak species.

From our experiment, we can conclude that the application of Mn fertilizer could potentially improve the oak seedling's field performance, which would enhance early seedling growth and increase oak forest regeneration success. Red maple's seed germination and seedling growth were depressed or less effected by Mn fertilizer treatments compared with oak. This could provide a more competitive advantage for oak seedlings in the early germination and growth processes.

Red maple is a more shade tolerant species than oak, and our greenhouse conditions provided an abundance of sunlight for oak which is different from the understory environment in the forest ecosystem. Additionally, our research focused on one Mn element concentration in different compound forms. More research in a forest setting and with other Mn concentrations to examine the effects on oak and maple germination and seedling growth is needed to further validate our findings.

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