GROWTH AND IRON SEQUESTERING OF PIN OAK *(QUERCUS PALUSTRIS)* SEEDLINGS INOCULATED WITH SOIL CONTAINING ECTOMYCORRHIZAL FUNGI

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Abstract. Ectomycorrhiza formation, biomass production, and iron accumulation were determined for pin oak *(Quercus palustris)* seedlings grown for 2 years in acidic (5.5 pH) or alkaline (7.5 pH) media and inoculated with soil from 1 of 3 sites: 1) a native pin oak forest, 2) an urban site with chlorotic pin oak trees, or 3) an urban site with non-chlorotic pin oak trees. Ectomycorrhiza formation on pin oak roots was similar for soil inocula from all 3 sites, similar for both pH treatments, and lacking on non-ectomycorrhizal seedlings that had been inoculated with autoclaved soil. Seedling biomass was greater in the acidic medium than the alkaline medium. Inoculated seedlings in the alkaline treatment had greater biomass than uninfected control seedlings inoculated with autoclaved soil in the alkaline treatment. The mean iron concentration of seedlings grown in the acidic medium (54.3 ppm) was significantly greater than that of seedlings grown in the alkaline medium (48.7 ppm). Inoculated seedlings had similar mean leaf iron concentrations despite pH differences (49.3 ppm under acidic conditions and 52.7 ppm under alkaline conditions). Mean leaf iron concentration was lowest for the uninoculated seedlings in alkaline medium (44.8 ppm), suggesting that ectomycorrhizae contribute to iron accumulation in pin oak under alkaline soil conditions. Results indicate that ectomycorrhizae influence both growth and iron sequestering under iron-limiting conditions and that some urban soils harbor infective pin oak ectomycorrhizal fungi.

Chlorosis commonly occurs in many species of plants including pin oaks *(Quercus palustris)* in urban areas when planted in calcareous, alkaline soil. Interveinal yellowing of leaves and whitening of leaf tissue in severe cases are characteristic symptoms of chlorosis. Iron deficiency is thought to be a primary reason for chlorosis in pin oak. Since iron is involved as a cofactor in the synthesis of chloroplast proteins and as a catalyst in photosystem redox reactions, a lack of sufficient iron results in decreased photosynthesis (3,17,40). Deficiencies in other micronutrients, including zinc and manganese, have also been associated with chlorosis in pin oak trees (26,27).

In urban soils iron is typically present, but often in forms unavailable for plant uptake due to binding to particles in alkaline soils (4). Oxidation of ferrous ion (Fe^{+2}) to ferric ion (Fe^{+3}) occurs readily in slightly acidic to alkaline soils (23,38). Ferric iron precipitates as ferric oxides, including the very insoluble ferric hydroxide ($Fe(OH)_3$). Ferric hydroxide has a solubility product of 10^{-38} M and the minimum iron concentration required for affecting plant iron nutrition is approximately 10^{-9} M (31). Root uptake of iron in higher plants is primarily, if not solely, as the ferrous ion (30,31). Ferrous iron is relatively available at a pH of 6 or below (32).

Various methods are currently used to treat pin oak chlorosis. Soil acidification, soil application of chelating agents and iron sulfate, tree injections and implants of ferric ammonium citrate or chelates, and foliar iron sprays have all been used to treat pin oak chlorosis caused by iron deficiency (14,27,29,37,43). These methods are not without problems and the treatments usually reduce the appearance of chlorosis for only a few months to 2–3 years. Soil acidification with sulfuric acid or elemental sulfur may require multiple applications to soils having a high calcium content. Both of these compounds can cause phytotoxic reactions in turf and other vegetation (35). Foliar application of iron sulfate and iron chelates acts quickly to reduce iron chlorosis. However, this method usually requires yearly applications (14). Application of ferric ammonium citrate or chelates through injections or implants has been reported to improve the color of pin oak leaves for up to 3 years. However, wounds from injections and implants cause wood discoloration, decay, and reduced tree energy storage areas and reserves if done improperly or repeatedly (37). Chelating agents applied to the soil increase the mobility of iron. Soil application of chelating agents is typically required every few years (14).

Mycorrhizal fungi are important symbionts of most plants and are associated with improved

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plant water relations, phosphorous uptake, and the increased uptake of iron, manganese, copper, and zinc in plants (13,41). Ectomycorrhizal fungi may increase the availability of iron through hydroxamate siderophores that they produce under iron-limiting conditions and which act as a chelating agent (34). There have been few investigations into the role that ectomycorrhizal fungi and their siderophores may play with respect to plant iron nutrition.

Lapeyrie (18) recently reviewed tolerance of plants to calcareous soil and classified plants into 3 groups: 1) those that tolerate calcareous soils termed calcicole plants, 2) calcifuge plants that are unable to tolerate calcareous soils, and 3) plants that tolerate calcareous soils only in association with microbes, including mycorrhizal fungi, termed symbiocalcicole. Austrian pine *(Pinus nigra nigricans),* aleppo pine *(Pinus halepensis),* and *Eucalyptus dumosa* trees that were grown in a calcareous soil medium and inoculated with ectomycorrhizal fungi exhibited less chlorosis than did the uninoculated plants (18). The reduced chlorosis of the inoculated plants was presumably from siderophore-sequestered iron uptake. Hence, it is possible that mycorrhizal symbiosis enhances iron uptake in some tree species, including pin oaks growing in alkaline soil.

It is not known whether urban soil systems differ in their mycorrhizal composition in comparison to natural forest settings. Urban soils often contrast sharply with surrounding native and relatively undisturbed soils (4,6), and have an elevated pH resulting from incorporation into the rooting zone of concrete rubble, crushed limestone, or calcareous subsoil. Urban soils also may have increased compaction, high contaminant levels from inorganic and organic compounds, elevated soil temperatures, mixed soil profiles, disrupted hydrologic flow, and interrupted nutrient and carbon cycles. Soil disruption has dramatically increased in the settled landscape since the advent of the bulldozer (4). Research has indicated that mass land perturbation, by means such as road building and soil storage piles from mining, greatly reduces the number of endomycorrhizal fungal propagules (7,28,36). The effects of massive soil disruption upon soil microbe populations and soil ecosystems are not fully understood. Thus, it is unknown whether altered and disturbed urban soils affect the mycorrhizal associations of urban trees. Further, there has been little research on mycorrhizal associations of trees in urban areas (8).

We investigated the effects of soil inoculations on the growth and iron sequestration of pin oak seedlings. We thought it possible that a decreased level of ectomycorrhizal association with pin oak trees in alkaline soils may partially explain pin oak chlorosis. Variation in ectomycorrhizal fungi among the 3 diverse sites was also investigated.

Methods and Materials

Stratified pin oak seeds were obtained in bulk from a commercial seed company (F.W. Schumacher Co., Inc., Sandwich, Massachusetts). Only undamaged seeds that sunk during a float test in water and that were within 20% of the mean weight of the bulk acorns were used. Seeds were surface sterilized on May 31, 1993, in a 30% (v/v) H_2O_2 solution for 15 minutes, washed afterwards 3 times in deionized H_2O (dH_2O), planted singly in each of 280 10-cm^3 pots containing pasteurized coarse vermiculite, and placed in a greenhouse with iron-free glass at the Plant Sciences Laboratory at the University of Illinois at Urbana/Champaign. Seedlings were grown for 2 months at 25 ± 4°C daytime and 18 ± 2°C nighttime temperatures, under ambient light conditions, watered daily with reverse osmosis-treated H_2O, and fertilized bimonthly with a 20:20:20 Peters brand fertilizer (nitrogen at 473 ppm) containing a full-strength, modified Hoaglands solution without iron (16). After 2 months, 120 seedlings of uniform size were selected for the study.

The experimental design was a 2:3:2 factorial: 1) acidic and alkaline media, 2) soil inoculum from 3 sites, and 3) inoculation with either natural or autoclaved soil inoculum. Seedlings were randomly distributed among the 12 treatments (10 seedlings per treatment combination) and transplanted into 1-gal plastic containers filled with a pasteurized 1:1:1 (v/v/v) peat moss:coarse vermiculite:calcined clay soil medium maintained under either acidic (pH = 5.5) or alkaline (pH = 7.5) regimes. The soil medium initially had a pH of approximately 5.5; thus,

no adjustment was required for the acidic treatment. The alkaline treatment soil was adjusted with 3 kg $CaCO_3$ per m^3 to produce a pH of 7.5. The soil medium was measured every 2 to 3 weeks throughout the study for pH changes with a Fisher Accumet 610 pH meter (Fisher Scientific, Pittsburgh, Pennsylvania) using a 1:1 soil:dH_2O slurry. The experimental soil media were adjusted to initial pH treatment levels every 3 to 5 weeks. No soil medium ever deviated more than 0.7 pH units from initial treatment pH levels. The pH of the acidic treatment increased with time and the soil medium pH was lowered in each container through the application of 250 mL of a 0.0014 M H_2SO_4 solution. The soil medium in the alkaline treatment decreased in pH with time, and the pH was increased by applying 300 mL of lime water that contained 1.18 g of $CaCO_3$ per L of reverse-osmosis deionized H_2O to each container.

The soil used for inoculation was collected from 3 different locations: 1) a pin oak stand at the Iroquois County (Illinois) Wildlife Area; 2) under 4 chlorotic pin oak trees on the campus of the University of Illinois at Urbana/Champaign; and 3) under 4 non-chlorotic pin oak trees on the campus of the University of Illinois at Urbana/Champaign. Soil at each site was collected by taking approximately 150 soil cores, 2 cm in diameter, to a 15 cm depth; the cores were subsequently bulked by site. Natural soil was collected 1 to 2 days prior to inoculating the artificial soil media. The soil pH values were 4.9 at the native pin oak stand, 7.0 at the chlorotic urban site, and 5.8 at the non-chlorotic urban site. Soil inoculum was incorporated at a 1:13 (v/v) inoculum to medium ratio. Soil that was autoclaved at 137°C for 2 hours was incorporated into the control containers at the same 1:13 (v/v) inoculum to medium ratio. Experimental seedlings had no sign of ectomycorrhizae prior to inoculation.

On August 3, 1993, inoculated seedlings were placed in a greenhouse room designed for plant and symbiotic microbe studies. The air supply was filtered through Air Guard type dp 2-40 filters (Air Guard Industries Inc., Louisville, Kentucky), ambient light was supplemented with Sylvania L41000 Metal Halide lamps (GTE Sylvania Inc., Manchester, New Hampshire) to maintain a 16-hour photoperiod with a minimum of 400 μmol/m^2/sec of photosynthetic photon flux, and seedlings were watered daily or as needed with water deionized by reverse osmosis. The greenhouse floor was washed with a commercial bleach solution containing sodium hypochlorite weekly to reduce the risk of contamination of the experimental seedlings by mycorrhizal fungi from soil on the floor. Temperatures were maintained at 25 ± 4°C daytime and 18 ± 2°C nighttime. Nutrients were supplied weekly with a Hoaglands solution (16) modified so that iron was applied as $FeCl_3$ at 100 ppm.

After the seedlings were 7 months old (5 months after inoculation), half of the seedlings from each treatment combination (n = 5) were randomly selected for harvesting to estimate biomass and ectomycorrhizal abundance, and half were overwintered for 3 months in a dormant state in a cold room set at 3–4°C. The soil moisture content of the overwintered seedlings was monitored and the seedlings were watered as needed to maintain a moist substrate. Ectomycorrhizal infection was assayed on 3 seedlings per treatment using the methods of Daughtridge et al. (9). Lateral roots were evenly divided into 4 groups, 1 of which was randomly selected for examination under a dissecting scope. Each lateral root was examined and considered infected if at least 1 ectomycorrhizal root tip was observed. The percentage of infection was determined by dividing the number of infected laterals by the total number of laterals and multiplying by 100. Biomass was determined from shoot and root tissue of 3 seedlings per treatment. Samples were dried for 48 to 72 hours at 75°C until a consistent dry mass was observed for each sample.

Winter-dormant seedlings were placed in a greenhouse on March 3, 1994, and grown for 2 additional months. Afterwards, 4 seedlings per treatment were harvested for biomass determinations and 5 seedlings per treatment for estimation of the concentration of iron in leaf tissue. Biomass was estimated as for previously harvested seedlings. Approximately 0.45 g (± 0.05 g) of leaf tissue was used to estimate plant iron concentrations. Tissue analyzed for nutrient concentration was ground in a Wiley mill to pass

through a 0.5 mm mesh screen and placed in 1.5 cc polyethylene vials that were rinsed 3 times in dH_2O, acid washed, and rinsed again 3 times in dH_2O. Neutron activation analysis (NAA) was performed using the University of Illinois TRIGA reactor. Samples and standard reference materials, from the National Institute of Standards and Technology (NIST), were placed into clean 1.5 cc polyethylene vials. The vials were irradiated at a neutron flux of 1.1×10^{12} n/cm^2/s for a period of 3 hours. After a 1-month decay, the samples were counted for 3 hours each using a hyperpure germanium detector. Elemental calibration was done using MST-certified solutions. The usual comparator method was used to calculate final concentrations. NAA values for Fe agreed very well with the NIST standard reference materials. Typical detection limits for Fe were 40 ppm.

Analysis of variance was used to test for significance of mean differences among the treatments at the 0.05 probability level. A Fisher's protected least significant difference was used to separate mean differences among treatments significant at the 0.05 probability level. Means significantly different at the 0.10 probability level were also discussed.

Results

Mycorrhizal infection. Pin oak seedlings inoculated with soil from either a native pin oak forest stand, beneath chlorotic urban pin oak trees, or beneath non-chlorotic urban pin oak trees had significantly more ectomycorrhizal infection 5 months after inoculation than seedlings inoculated with autoclaved soil inoculum (Table 1). Between 71% and 97% of the lateral roots of seedlings grown with soil inoculum contained at least 1 ectomycorrhizal root tip. The control pin oak seedlings inoculated with the corresponding autoclaved soil inoculum had no ectomycorrhizal infection except for 1 seedling that had a single ectomycorrhizal root tip observed on a lateral root. The acidic and alkaline treatments exhibited no discernible difference in ectomycorrhizal infection.

Pin oak seedlings examined after the second growth season were visually assayed without magnification (data not shown). Seedlings inoculated with soil inoculum had abundant ectomycorrhizae,

Table 1. The ectomycorrhizal infection of lateral roots of pin oak *(Quercus palustris)* seedlings grown in either acidic or alkaline medium and inoculated with live or autoclaved soil from 3 different sites.

Soil pH[1]	*Site*[2]	Autoclaved *inoculum*	% lateral *root infection*
Acidic	Forest	No	77.7 ab[3]
Acidic	Chlorotic	No	88.9 ab
Acidic	Non-chlorotic	No	87.2 ab
Acidic	Forest	Yes	0.0 c
Acidic	Chlorotic	Yes	0.0 c
Acidic	Non-chlorotic	Yes	1.7 c
Alkaline	Forest	No	77.3 ab
Alkaline	Chlorotic	No	71.4 b
Alkaline	Non-chlorotic	No	96.6 a
Alkaline	Forest	Yes	0.0 c
Alkaline	Chlorotic	Yes	0.0 c
Alkaline	Non-chlorotic	Yes	0.0 c
ANALYSIS OF VARIANCE			
Model			**0.0001**[4]
Ph			0.7367
Site			0.4220
Inoculation			**0.0001**
pH*Site			0.5778
pH*Inoculation			0.8215
Site*Inoculation			0.5073
pH*Site*Inoculation			0.5066

[1]Acidic soil pH = 5.5 and alkaline soil pH = 7.5.
[2]Soil inoculum from pin oak forest stand, urban chlorotic pin oak trees, and urban non-chlorotic pin oak trees.
[3]Means (n = 36) in the same column with a different letter are significantly different at the $\alpha = 0.05$ level using a Fisher's protected LSD.
[4]Significance probabilities from the F statistic from an ANOVA. Significant values ($P \leq 0.05$) in bold.

and no discernible differences were observed in ectomycorrhizal abundance with respect to pH of the medium or source of the soil inoculum. However, both white and dark ectomycorrhizae were observed on the lateral roots in the urban soil inoculum treatments, while only dark ectomycorrhizae were observed on roots of seedlings inoculated with soil from the native pin oak forest, suggesting different mycorrhizal populations in the urban soil than the forest soil. Pin oak seedlings inoculated with autoclaved soil inoculum remained free of ectomycorrhizae through the second growing season under our experimental conditions.

First year biomass. Root biomass differed significantly among treatments after 7 months (Table 2). Differences were associated with the medium pH, the site from which soil inoculum was

Table 2. Biomass partitioning and root to shoot ratio of 7-month-old pin oak *(Quercus palustris)* seedlings grown in either acidic or alkaline soil medium and inoculated with live or autoclaved soil from 3 different sites.

Soil pH[1]	Site[2]	Autoclaved inoculum	Shoot mass(g)	Root mass(g)	Total mass(g)	Root/ shoot
Acidic	Forest	No	1.63 a	5.32 ab[3]	6.97 a	3.23 a
Acidic	Chlorotic	No	1.73 a	5.31 ab	7.06 a	3.01 a
Acidic	Non-chlorotic	No	2.00 a	5.57 a	7.60 a	2.99 a
Acidic	Forest	Yes	1.13 a	3.09 cd	4.27 a	2.62 a
Acidic	Chlorotic	Yes	1.73 a	3.33 bcd	5.09 a	2.03 a
Acidic	Non-chlorotic	Yes	2.03 a	4.68 abc	6.77 a	2.31 a
Alkaline	Forest	No	1.13 a	2.15 d	3.34 a	1.92 a
Alkaline	Chlorotic	No	1.53 a	3.41 bcd	4.98 a	2.22 a
Alkaline	Non-chlorotic	No	1.73 a	4.40 abc	6.18 a	2.47 a
Alkaline	Forest	Yes	1.37 a	3.28 bcd	4.69 a	2.22 a
Alkaline	Chlorotic	Yes	1.57 a	4.17 abcd	5.80 a	2.66 a
Alkaline	Non-chlorotic	Yes	1.57 a	4.54 abc	6.16 a	2.76 a
Means			1.60	4.10	5.74	2.54
ANALYSIS OF VARIANCE						
Model			0.4057[4]	**0.0459**	0.0608	0.4138
pH			0.1616	**0.0376**	0.0411	0.1718
Site			0.0411	**0.0415**	0.0233	0.8376
Inoculation			0.7018	0.2222	0.2834	0.3782
pH*Site			0.8183	0.5821	0.7613	0.2738
pH*Inoculation			0.5549	**0.0074**	0.0196	0.0247
Site*Inoculation			0.9273	0.9705	0.9798	0.9796
pH*Site*Inoculation			0.4652	0.4914	0.4377	0.8844

[1]Acidic soil pH = 5.5 and alkaline soil pH = 7.5.
[2]Soil inoculum from pin oak forest stand, urban chlorotic pin oak trees, and urban non-chlorotic pin oak trees.
[3]Means (n = 36) in the same column with a different letter are significantly different at the $\alpha = 0.05$ level using a Fisher's protected LSD.
[4]Significance probabilities from the F statistic from an ANOVA. Significant values ($P \leq 0.05$) in bold.

Table 3. Biomass partitioning and root to shoot ratio of 2-year-old pin oak *(Quercus palustris)* seedlings grown in either acidic or alkaline soil medium and inoculated with live or autoclaved soil from 3 different sites.

Soil pH[1]	Site[2]	Autoclaved inoculum	Shoot mass(g)	Root mass(g)	Total mass(g)	Root/ shoot
Acidic	Forest	No	7.83 a[3]	8.41 ab	16.23 a	1.09 a
Acidic	Chlorotic	No	5.83 ab	7.92 abc	13.76 ab	1.36 a
Acidic	Non-chlorotic	No	7.46 a	8.24 ab	15.69 a	1.13 a
Acidic	Forest	Yes	7.94 a	8.43 ab	16.37 a	1.05 a
Acidic	Chlorotic	Yes	5.12 abcd	6.95 abc	12.07 ab	1.39 a
Acidic	Non-chlorotic	Yes	7.19 a	10.33 a	17.51 a	1.47 a
Alkaline	Forest	No	5.60 abc	7.50 abc	13.09 ab	1.35 a
Alkaline	Chlorotic	No	3.36 bcd	5.47 bcd	8.83 bc	1.72 a
Alkaline	Non-chlorotic	No	5.60 abc	6.96 abc	12.55 ab	1.46 a
Alkaline	Forest	Yes	2.19 d	3.24 d	5.43 c	1.50 a
Alkaline	Chlorotic	Yes	3.79 bcd	4.79 cd	8.49 bc	1.35 a
Alkaline	Non-chlorotic	Yes	2.65 cd	3.17 d	5.82 c	1.10 a
Means			5.38	6.78	12.15	1.33
ANALYSIS OF VARIANCE						
Model			**0.0012**[4]	**0.0021**	**0.0010**	0.0545
pH			**0.0001**	**0.0001**	**0.0001**	0.0541
Site			0.1421	0.5393	0.2936	0.1089
Inoculation			0.0658	0.0687	0.0550	0.6066
pH*Site			0.3650	0.5329	0.5157	0.1914
pH*Inoculation			0.1680	**0.0199**	**0.0472**	0.0761
Site*Inoculation			0.5098	0.6861	0.6551	0.5092
pH*Site*Inoculation			0.2494	0.1813	0.1943	0.0887

[1]Acidic soil pH = 5.5 and alkaline soil pH = 7.5.
[2]Soil inoculum from pin oak forest stand, urban chlorotic pin oak trees, and urban non-chlorotic pin oak trees.
[3]Means (n = 36) in the same column with a different letter are significantly different at the $\alpha = 0.05$ level using a Fisher's protected LSD.
[4]Significance probabilities from the F statistic from an ANOVA. Significant values ($P \leq 0.05$) in bold.

collected, and an interaction between pH and inoculation. Seedlings grown in the acidic substrate treatments had greater root biomass (4.55 g) than those grown in the alkaline substrate treatments (3.66 g). Seedlings inoculated with soil from beneath non-chlorotic urban pin oak trees had greater root biomass (4.80 g) than seedlings inoculated with soil from a pin oak forest stand (3.46 g). Root biomass in seedlings inoculated with soil from beneath chlorotic pin oak trees was intermediate (4.06 g). Root biomass was greatest in seedlings inoculated with live soil inoculum and grown in acidic medium (5.40 g) and less in seedlings grown in an acidic medium inoculated with autoclaved soil (3.71 g), alkaline medium inoculated with live inoculum (3.32 g), and alkaline medium inoculated with autoclaved inoculum (4.00 g). Mean total biomass of root and shoot tissue differed significantly among treatments at the 0.10 probability level ($p = 0.061$). Total biomass patterns, as influenced by soil media, inoculation, and the source of soil inoculum, were similar to root biomass patterns.

Second year biomass. Shoot, root, and total biomass differed significantly among the treatments after the second growing cycle (Table 3). The pH of the medium had a significant influence on biomass accumulation. Seedlings grown in the acidic medium had more root (8.38 g), shoot (6.89 g), and total (15.27 g) biomass than the root (5.17 g), shoot (3.87 g), and total (9.04 g) biomass of the seedlings grown in the alkaline medium. A pH and inoculation interaction occurred for root and total biomass. Inoculated and control seedlings grown under acidic substrate conditions had similar biomass. In contrast, root and total biomass

were less in seedlings grown with autoclaved inoculum in the alkaline treatment than for the seedlings in the inoculated alkaline treatment. However, biomass was similar for seedlings inoculated with autoclaved control and live soil from the chlorotic site and grown in the alkaline medium. Mean biomass accumulation in shoot, root, and total tissue differed significantly at the 0.10 probability level ($p = 0.055$ to 0.069) between seedlings grown with either live or autoclaved inoculum. Seedlings inoculated with live inoculum had more root (7.42 g), shoot (5.95 g), and total (13.36 g) biomass than the root (6.14 g), shoot (4.81 g), and total (10.95 g) biomass in seedlings inoculated with autoclaved inoculum. The root to shoot ratio (R:S) was significantly different at the 0.10 probability level ($p = 0.054$). Seedlings grown in the acidic medium had a lower R:S (1.25) than those grown in the alkaline medium (1.41). A pH and inoculation interaction occurred for the R:S variable at the 0.10 probability level ($p = 0.076$), in that seedlings inoculated with live inoculum and seedlings grown in the autoclaved acidic medium had a similar R:S. In contrast, the R:S differed between seedlings inoculated with live or autoclaved inoculum for seedlings grown in the alkaline medium.

Iron leaf tissue concentration. The mean iron concentration differed significantly between soil pH treatments (Table 4). The iron concentration was 54.3 ppm in seedlings grown in an acidic medium and 48.7 ppm in seedlings grown in alkaline medium. A significant pH and inoculation interaction occurred. The iron concentration of the control treatments was higher in the acidic seedlings (59.3 ppm) than the alkaline seedlings (44.8 ppm). In contrast, the iron concentration was similar for seedlings infected by live inoculum in either an acidic (49.3 ppm) or alkaline (52.7 ppm) medium.

Table 4. The concentration of iron in leaf tissue from 2-year-old pin oak *(Quercus palustris)* seedlings grown in either acidic or alkaline soil medium and inoculated with live or autoclaved soil from 3 different sites.

Soil pH[1]	Site[2]	Autoclaved inoculum	Iron (ppm)
Acidic	Forest	No	53.1 abcde[3]
Acidic	Chlorotic	No	48.7 bcde
Acidic	Non-chlorotic	No	46.1 cde
Acidic	Forest	Yes	54.1 abcd
Acidic	Chlorotic	Yes	65.0 a
Acidic	Non-chlorotic	Yes	58.7 ab
Alkaline	Forest	No	56.1 abc
Alkaline	Chlorotic	No	55.2 abc
Alkaline	Non-chlorotic	No	46.7 bcde
Alkaline	Forest	Yes	51.3 bcde
Alkaline	Chlorotic	Yes	40.7 e
Alkaline	Non-chlorotic	Yes	42.5 de
Means			5.15
ANALYSIS OF VARIANCE			
Model			**0.0137**[4]
Ph			**0.0336**
Site			0.2365
Inoculation			0.6769
pH*Site			0.2998
*pH*Inoculation*			***0.0010***
Site*Inoculation			0.6136
pH*Site*Inoculation			0.1450

[1]Acidic soil pH = 5.5 and alkaline soil pH = 7.5.
[2]Soil inoculum from pin oak forest stand, urban chlorotic pin oak trees, and urban non-chlorotic pin oak trees.
[3]Means (n = 36) in the same column with a different letter are significantly different at the $\alpha = 0.05$ level using a Fisher's protected LSD.
[4]Significance probabilities from the F statistic from an ANOVA. Significant values ($P \leq 0.05$) in bold.

Discussion

Foliar iron concentrations were higher in seedlings grown in the alkaline substrate and infected with ectomycorrhizae by inoculation with soil than in the control seedlings grown without ectomycorrhizae in the alkaline treatment. The inoculated seedlings in both pH treatments had a similar tissue iron concentration. This suggests that ectomycorrhizal fungi may increase the iron concentration of pin oak trees under alkaline conditions. Ectomycorrhizal fungi probably increase iron concentration in other plants under iron-limiting conditions (15,18,19,21,22,34). For example, Clement et al. (1977, as cited in 13) and Le Tacon (20) discovered that Austrian pine seedlings grown in calcareous media were sometimes chlorotic when ectomycorrhizae were absent. In comparison, no ectomycorrhizal Austrian pine trees were chlorotic and a genetic tolerance was ruled out. Mycorrhizal root mats have also been correlated with higher soil iron concentrations in comparison with soil locations not containing these mats (11).

Inoculation of the media with soil containing ectomycorrhizae clearly enhanced growth of pin

oak seedlings in comparison to control seedlings. This indicates that there are benefits to pin oak seedlings from ectomycorrhizal symbioses. Thus, ectomycorrhizae may have contributed to enhanced growth of the seedlings grown in alkaline substrate. However, seedlings in an alkaline medium and inoculated with soil from the urban chlorotic pin oak site and control seedlings inoculated with soil from this site had similar mean biomass values, suggesting that ectomycorrhizal populations at this site may have been less effective in influencing plant growth than ectomycorrhizal fungi from the other sites tested. The ectomycorrhizal symbiont *Pisolithus tinctorius* has been demonstrated to increase the growth of pin oak in controlled experiments (1,24). *Pisolithus tinctorius* has also been found associated with pin oak trees in urban areas (25). Vesicular arbuscular mycorrhizae (VAM) also form on pin oak (42), though we did not determine the VAM status of the experimental pin oak seedlings.

Lapeyrie (18) recently reviewed the tolerance of woody plants growing in calcareous soil and cited 4 studies in which seedlings grown in sterile, calcareous media were chlorotic. A reduction in chlorosis and mortality occurred in some cases after inoculation with ectomycorrhizal fungi. The health of inoculated seedlings on calcareous media was similar to seedlings on sterile acidic media. It has been demonstrated that ectomycorrhizal fungi produce hydroxamate siderophores (39). Siderophores have been associated with an increased availability of iron for plants (2,5,12,33).

Acidic soil medium favored a higher iron concentration in pin oak leaf tissue than did the alkaline treatment. Iron is more mobile in acidic soil systems than in alkaline systems (32). Ferric iron is more commonly associated with alkaline soil systems and readily precipitates into unavailable forms with low solubility (38). Biomass was also consistently greater in pin oak seedlings grown under acidic conditions during the first and second years of growth. These findings are consistent with observations that the native soil habitat of pin oak is acidic (10).

We found no differences among soils from the 3 sites in their capacity to form ectomycorrhizal roots on pin oak seedlings. The taxa of ectomycorrhizae were not determined so we do not know which specific fungi colonized the root systems in the various treatments. Mycorrhizal fungi are known to have wide to narrow ranges of pH tolerance (13). The observations in this study that seedlings inoculated with urban soils had both light and dark colored ectomycorrhizae, while the pin oak forest soil inoculum produced dark ectomycorrhizae, and that alkaline soil from the chlorotic urban pin oak site did not increase biomass of inoculated seedlings compared with autoclaved soil inoculum from the same site suggests that ectomycorrhizal taxa differ in occurrence among the sites.

Conclusions

Results of this study clearly demonstrate that pin oak-infective ectomycorrhizal propagules occurred in both an acidic and an alkaline urban soil from under pin oaks. Ectomycorrhizae were associated with increased iron uptake and growth of seedlings after 2 growing seasons in an alkaline soil, but not an acidic soil, suggesting the importance of ectomycorrhizae in improving the iron nutritional status of pin oak trees in alkaline soils. It is possible that pin oak chlorosis is mitigated in many alkaline urban soils by ectomycorrhizae. Alternatively, one could speculate that chlorosis of pin oak trees is more pronounced on alkaline soils lacking ectomycorrhizal fungi. The determination of such a pattern is possible with sampling of pin oak roots for ectomycorrhizal assessment and analysis of pH and other properties of associated soils across a wide range of urban sites.

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Résumé. Une évaluation de la formation d'ectomycorrhizes, de la production de biomasse et de l'accumulation en fer dans des semis de chênes des marais (*Quercus palustris*) inoculés, et mis en croissance dans des substrats acide (pH: 5,5) et alcalin (pH: 7,5), a été réalisée à partir de trois provenances de sol: 1) un sol d'une forêt naturelle de chênes des marais, 2) un sol d'un site urbain planté de chênes des marais à feuilles chlorotiques, et 3) un sol d'un site urbain planté de chênes des marais à feuilles sans chlorose. La biomasse produite par les semis a été plus forte dans le substrat acide que dans celui alcalin. Les semis inoculés dans le substrat alcalin ont produit une biomasse supérieure à celle des semis contrôle. L'accumulation de fer dans les semis croissant dans le substrat acide a été significativement supérieure à celle des semis en substrat alcalin. Les concentrations foliaires de fer ont été les moindres avec les semis non inoculés dans le substrat alcalin.

Zussammenfassung. Hier wurden die Entwicklung von Ectomycorrhizen, die Produktion von Biomasse und die Akkumulation von Eisen bei Sumpfeichensämlingen bestimmt, die in saurem (pH5,5) und basischem (pH7,5) Medium vorgezogen und mit drei verschiedenen Böden (1. ein natürlicher Sumpfeichenboden, 2. von einem urbanen Standort mit chlorotischen Sumpfeichen und 3. von einem urbanen Standort mit nicht-chlorotischen Bäumen) geimpft wurden. Die Biomasse der Sämlinge in saurem Medium war größer als in dem basischen Medium. Die geimpften Sämlinge im basischen Medium hatten eine höhere Biomasse als die Kontrollsämlinge. Die Eisenakkumulation in den auf saurem Milieu gezogenen Sämlingen war wesentlich größer als in den auf basischen Medium gezogenen. Die Eisenakkumulation in den Blättern war in den ungeimpften Sämlingen in basischem Milieu am niedrigsten.