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Effects of flooding regime, mycorrhizal inoculation and seddling treatment type on first-year survival of nuttall oak (Quercus nuttallii PALMER)

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EFFECTS OF FLOODING REGIME, MYCORRHIZAL INOCULATION AND SEEDLING TREATMENT TYPE ON FIRST-YEAR SURVIVAL OF NUTTALL OAK (*Quercus nuttallii* PALMER)

Virginia Burkett and Hans Williams[1]

Abstract—Three different types of Nuttall oak (*Quercus nuttallii* Palmer) seedlings were planted on floodprone, former cropland in Mississippi, Louisiana, and Texas. The three types of 1+0 seedlings planted at each site in January and February of 1995 were bareroot seedlings, seedlings grown in 164 square centimeters plastic containers, and container-grown seedlings inoculated with vegetative mycelia of *Pisolithus tinctorious* (Pers.) Coker and Couch. Seedlings at the Mississippi site were planted in a split-plot design at three different elevations, which provided three different natural flooding treatments. Seedlings at the other two sites were planted in a Latin square design at a single elevation. Significant differences in the survival and condition of the seedlings during the first growing season were observed at the Louisiana site, favoring the inoculated container-grown seedlings over the other two stock types. First-year seedling survival at the site in Texas, which had the best drainage of the three sites, was not significantly different between treatments. Small mammals clipped 98 percent of the container-grown seedlings at the Mississippi site.

INTRODUCTION

Bottomland hardwood forested wetlands are characteristically exposed to soil saturation and periodic or continuous flooding at various times of the year. Depth to the permanent water table and timing, frequency, and duration of flooding play key roles in the occurrence and growth rate of hardwood species from seed germination, in early seedling survival, and in growth during establishment (Kennedy and Johnson 1984). Seedling survival can be strongly influenced by root morphology, which affects nutrient and water accumulation. Oak seedlings grown in containers develop more primary lateral roots and secondary roots than bareroot seedlings (Dixon and others 1981a).

The roots of forest seedlings are commonly ingrown with mycorrhizal fungi that penetrate the surrounding soil and provide access to a much greater soil volume than uninfected roots. The presence of mycorrhizal fungi may be especially important to seedling growth on harsher sites (Read 1991), because they enhance the transport of nutrients, water, and organic materials to the seedling. Dixon and others (1981a, b) found that leaf area, number of primary lateral roots, and carbohydrate reserves were increased in container-grown black oak seedlings that were inoculated with *Pisolithus tinctorious,* (Pers.) Coker and Couch, a common mycorrhizal fungus associated with many southern forest species.

Pisolithus tinctorius, an "ectomychorrizal" fungus which forms a mantle or sheath on the root surface, has been used effectively to enhance pine (*Pinus*) seedling growth and survival (Marx and others 1977). Mycorrhizal fungi are obligate plant symbionts; flooding, cropping, and fallow have been shown to reduce their populations. Despite the assumption that wetland plants are non-mycotrophs, wetland species generally develop mycorrhizae when soils become relatively dry (Allen 1990). The influence of seasonal flooding and soil anoxia on mycorrhizal development and functions in hardwood forests is poorly understood.

The purpose of this study was to determine the relative importance of seedling type and mycorrhizal inoculation in the growth of Nuttall oak seedlings for wetland reforestation.

METHODS

Seedling Culture

Common nursery practices were used to cultivate the container and bareroot seedlings used in these experiments. Thirty pounds of Nuttall oak seed were obtained from a seed source in the Delta area of Mississippi in late April of 1994 and placed in cold storage at 1.7 °C. The acorns were soaked overnight in a 5-gallon bucket of tap water; 111 acorns that floated were discarded. The remainder were placed in 18 plastic bags and refrigerated at 1.7 °C. Bags were turned and opened to check for spoilage three times each week through May 26, 1994, when 2,222 acorns were individually sown in 164-centimeter^3 plastic seedling cones (Ray Leach "Cone-tainer" Nursery, Canby, Oregon).

A 1:1 homogeneous mixture of autoclaved peat moss and construction-grade vermiculite was used as a planting medium for the container stock. A total of 2,222 seeds were sown, thoroughly watered, and placed in a greenhouse at Stephen F. Austin State University. By July 18, a total of 1928 container seedlings had been produced and moved into an outdoor shadehouse. They were fertilized biweekly and watered as needed.

On July 6, 1994, every other tray of seedlings in the shadehouse was removed and inoculated with vegetative mycelial inoculum of *P. tinctorius.* One-half (5 grams of

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fungal mycelia) of a commercially available inoculum kit was applied in a drench following procedures recommended by the distributor (Mycorr Tech, Inc., Pittsburgh, PA). Seedlings were inoculated again on August 30 and December 21, 1994. The inoculated seedlings were maintained on separate tables in the shadehouse but otherwise treated the same as the noninoculated stock.

Bareroot seedlings (46 centimeters or larger) were obtained from the same seed source and seed lot as the container-grown seed.

Morphological Analysis Prior to Planting

On January 5, 1995, 50 seedlings of each container seedling type (container with inoculation and container without inoculation) were selected using a random numbers table. They were gently removed from their containers and soaked in tap water for 1 hour to loosen the potting medium. After washing, the exposed root systems were submerged in tap water overnight so that they would not dry out.

For the preplanting comparison of bareroot Nuttall oak seedlings with those grown in containers, 250 bareroot seedlings were obtained from the seed/seedling source in Mississippi. They were lifted from the nursery bed on January 3, 1995, and transported overnight in sealed shipping bags to Stephen F. Austin State University, where they were placed in cold storage at 1.7 °C. On January 6, 1995, 50 bareroot seedlings and the container seedlings that were harvested the prior day were brought into the laboratory for morphological and biomass measurements. Weight of aboveground and belowground plant material was measured using a Mettler PC 8000 analytical balance (Mettler Instrument Corp., Heightstown, NJ). Root volume was measured using a displacement procedure described by Burdett (1979). Stems and roots were placed in a drying oven at 60 °C. When a constant weight was reached, the dry weights of stem and root tissue were recorded.

For all statistical analyses used in these experiments the level of significance was established at $\alpha = 0.05$. A one-way analysis of variance (ANOVA; using Proc GLM of the Statistical Analysis System, SAS Institute Inc. Cary, NC) was used to test for fixed treatment effects on seedling morphology prior to planting. If significant differences were found between at least two of the three seedling types, means were compared using Duncan's multiple range test (Freund and Wilson, 1993).

Field Trials

Yazoo National Wildlife Refuge, MS—Field experiments that involved the testing of seedling treatment and flooding regime were conducted at Yazoo National Wildlife Refuge (NWR) in the Delta Region of west-central Mississippi. The planting site was located on a tract of farmed wetlands that was recently annexed to the Yazoo NWR in Sharkey County, MS, 2.5 kilometers east of the community of Anguilla. A 1994 soil survey of the tract indicated that the soil type at this site is Sharkey clay (very fine, montmorillonitic, nonacid, thermic Vertic Haplaquept). The Sharkey soil contains montmorillonitic clay that, when dry, develops cracks that are from 2 to 10 centimeters wide and several centimeters deeper than wide (Soil Conservation Service 1962).

The field is roughly rectangular in shape, with a manmade drainage ditch running the entire length of the east side, from north to south along its long axis. The field has a very gentle slope (< 2 percent) dipping to the east, toward the drainage ditch. An RDS WL-80 water level recorder (Remote Data Systems, Inc., Wilmington, NC) was placed near the study plots on a wooden pile on the west side of the drainage ditch.

An elevation survey of the field was conducted to identify three elevation contours that would best represent three natural flooding regimes (i.e., whole plots): frequently flooded, less frequently flooded, and not flooded. The three elevations relative to mean sea level are 28.1 meters, 28.8 meters, and 29.4 meters. Four 22.8-meter by 7.6-meter whole plots (i.e., replications) were laid out lengthwise along each of the three elevations, with a 7.6-meter spacing between each plot. Each whole plot was subdivided into three 7.6-meter by 7.6-meter subplots, oriented parallel to the elevation gradient. Each of the three seedling treatments was assigned at random to one of the subplots or "split plot" within each whole plot.

Container seedlings were transported in a covered van from the university to the site on January 9, 1995. Bareroot seedlings were lifted from the nursery bed on January 9 and transported in sealed shipping bags directly to the planting site at Yazoo NWR on January 10, 1995. Thirty seedlings were planted with a planting shovel on January 10 and 11, 1995, in each subplot on a 1.5-meter by 1.5-meter spacing arrangement.

Plant competition at each of the three elevations on August 19, 1995, was compared by describing the percentage cover by species and overall height in a 1-square meter plot located between each whole plot at each elevation.

Alazan Bayou Wildlife Management Area, TX—The Alazan Bayou Wildlife Management Area (WMA), owned and managed by the Texas Parks and Wildlife Department (TP&W), is located in northeastern Texas, approximately 10 miles north of Lufkin in Nacogdoches County. The WMA includes approximately 800 hectares of former pasture and bottomland hardwood forests, mostly located along Alazan Bayou. A relatively low and flat mowed area on the southern end of the tract was selected for the field trials at Alazan Bayou WMA. The 1980 soil survey of Nacogdoches County indicates that the site is Mantachie sandy loam (fine loamy, silicious, acid thermic Aeric Fluvaquent; Dolezel and Fuchs 1980).

There was only one main factor of interest at the Alazan Bayou site: seedling treatment at three fixed qualitative levels (bareroot, container, and container with inoculation). Three 22.8-meter by 7.6-meter blocks (i.e., reps), numbered 1, 2, and 3, were laid out in a north-to-south direction. Each of the blocks was split into three equal size plots (7.6 meters by 7.6 meters), oriented in an east-to-west direction. For Block 1, each of the seedling treatments was randomly assigned to the plots. The seedling treatment assigned to the westernmost plot of Block 1 was then assigned to the middle plot of Block 2; the seedling treatment assigned to the middle plot of Block 1 was assigned to the easternmost plot of Block 2; and so forth, creating a 3 by 3 Latin Square design. The order of experimentation was restricted, that is, blocked in two directions: rows and columns.

Ninety bareroot seedlings were selected at random from those that had been placed in cold storage at the university on January 4, 1995. Ninety container seedlings of each treatment type were selected at random from those that remained in the shadehouse. Seedlings were planted with planting shovels in rows in each plot on a 1.5-meter by 1.5-meter spacing arrangement on February 9, 1995.

To compare the morphology of seedlings at the end of the first growing season, three randomly selected seedlings were dug up from each of the nine plots with a planting shovel on January 30, 1996. The procedures that were used to measure all variables but root area were the same as those used in the preplanting analysis of seedlings. For root area measurements, lateral roots were removed with a small scalpel and scanned with the Li-Cor Portable Area Meter (LI-3000 A, Lincoln, NE).

Bayou Macon Wildlife Management Area, LA—Bayou Macon WMA in northeast Louisiana, owned and managed by the Louisiana Department of Wildlife and Fisheries, is located east of Bayou Macon between State Highways 2 and 582, approximately 15 kilometers west of the Mississippi River. The predominant cover type is bottomland hardwood forest, but 644 hectares had been clearcut and used for agricultural purposes before the land was purchased by the State. The soil type at the Bayou Macon WMA planting site is Sharkey clay[2] (Allen and others 1988).

The experimental design at the Bayou Macon WMA was the same as that used at the Alazan Bayou site in Texas. Seedling treatment was the only factor of interest. Seedlings were planted with planting shovels on December 14, 1995.

Data analyses—Analysis of variance (using Proc GLM, SAS) was used to test for treatment effects on seedling height and other continuous response variables measured in-situ at each site. The responses were transformed, if necessary, to meet the assumptions of homogeneity for the error terms of the model. If significant differences were found between treatments, means that involved two populations (e.g., flooded and nonflooded plots) were compared using the least significant difference or LSD test (Fisher 1960). Duncan's multiple range test was used to compare means from three different populations (e.g., three different seedling types).

For the analysis of the morphology of the 1+1 seedlings harvested at Alazan Bayou WMA, a multivariate analysis of variance (MANOVA, SAS) was performed on those responses which appeared correlated; otherwise, a univariate analysis was performed. Prior to MANOVA, each variable was tested for homogeneous variance assumptions and normality. Means were compared using Duncan's multiple range test when significant differences were found.

Categorical data analyses (using Proc Catmod, SAS) were used to compare rates of survival, dieback, herbivory, and basal sprouting among seedling treatments and flooding regime. If normality assumptions were not met by the categorical model, a binomial test of proportions was used to test for differences between treatments. Contrasts were used to compare means where significant differences were found.

RESULTS

Morphological Differences Prior to Planting

All morphological measurements indicated significant differences between the 1+0 bareroot and the container seedlings prior to planting (table 1). The bareroot seedlings had a significantly larger stem biomass and root collar diameter than the inoculated and noninoculated container seedlings. The initial height of the average bareroot seedling was approximately 10 percent higher than that of the container seedlings. The root systems of the bareroot seedlings were also significantly larger than those of the container seedlings, but the container seedlings had roughly twice as many primary lateral roots. The only significant morphological difference found between the two container seedling treatments prior to planting was the number of primary lateral roots greater than 0.5 millimeters. The inoculated container seedlings had 17 percent more primary lateral roots than the container seedlings that were not inoculated (table 1).

Table 1—Morphology of the three types of Nuttall oak seedlings prior to planting at the three field sites (means within a row having a common superscript are not significantly different, $\alpha = 0.05$)

Inoculated variable	Bareroot	Container	Container
Stem height (cm)	70.4[a]	60.3[b]	65.6[b]
Stem dry weight (g)	12.95[a]	4.52[b]	4.65[b]
Root dry weight (g)	11.30[a]	7.51[b]	7.41[b]
Stem diameter at root collar (mm)	13.0[a]	9.0[b]	9.0[b]
Root volume (ml)	11.55[a]	3.50[b]	3.71[b]
Primary lateral roots	16[c]	30[b]	35[a]

[2] Personal communication. June 17, 1996. Floyd Hooker, NRCS, Lake Providence, LA.

Yazoo National Wildlife Refuge

The planting site at Yazoo NWR was partially flooded twice during the first growing season. The lowest elevation, 28.1 meters mean sea level, was flooded continuously from March 8 to March 23, 1995 and again from April 26 to May 1, 1995. This flooding exposed the seedlings at the lowest elevation to 21 days of flooding. The middle planting elevation was flooded for only five days (March 16 to 20) during the first flood event and seven days (April 24 to 30) during the second event. The highest planting elevation, 29.4 meters mean sea level, did not flood during either event.

The predominant plant species that naturally invaded the planting site at the lowest elevation was *Iva annua*, which covered an average 75 percent of three 1-square meter plots sampled on August 17, 1995. No other species had more than 5 percent cover at any elevation in the canopy of the competing vegetation at the lowest elevation. Competition in the middle plots was more diverse, with *Iva annua* and *Sorghum halepense* (Johnson grass) occupying 45 percent and 25 percent of the upper canopy, respectively. The upper plots were heavily dominated by a dense growth of Johnson grass, which covered an average of 90 percent of each meter quadrant sampled.

Early in the first growing season (June 1, 1995) average survival (aboveground) of both types of container-grown seedlings exceeded 96 percent at all three elevations. At that time, survival of the bareroot stock averaged 45 percent, 27 percent, and 31 percent at the highest, middle, and lowest elevations, respectively. Survival through the end of the first growing season was difficult to compare among treatments because only 18 of the 720 container-grown seedlings had not been clipped by rodents. The container seedlings at the higher elevation were the first to be clipped, probably because there was more protective cover for rodents under the dense growth of Johnson grass. *Iva annua*, which dominated the competition at the lower elevation, reached comparable height but has a canopy structure that allows much higher light penetration at ground level.

Alazan Bayou Wildlife Management Area, TX

The survival and development of basal sprouts in seedlings during the first growing season at Alazan Bayou was not significantly different among the three seedling treatments (table 2). The categorical data analysis did detect significant effects of seedling treatment on shoot dieback, which was generally confined to the upper 10 centimeters of the stem. One third of the bareroot seedlings experienced partial dieback, compared to an average 67 percent dieback in both types of container stock (table 2).

Live stem length was significantly higher in the container-grown seedlings, even though they were more prone to partial shoot dieback (table 3). Root biomass and stem diameter were significantly higher in the bareroot seedlings. The inoculated container seedlings had a significantly

Table 2—Percentage of seedlings that survived, exhibited basal sprouts, and partially died back at the Alazan Bayou WMA in NE Texas during the first growing season (means within a row having common superscript are not significantly different, $\alpha = 0.05$)

Variables	Bareroot	Container	Inoculated container
Survival	94.08[a]	97.31[a]	97.31[a]
Basal sprout	5.91[a]	1.61[a]	2.80[a]
Partial dieback	33.33[b]	66.66[a]	68.18[a]

Table 3—Morphology and biomass of seedlings after first growing season at Alazan Bayou WMA in NE Texas (means within a row having same superscript are not significantly different, $\alpha = 0.05$)

Variables	Bareroot	Container	Inoculated container
Stem length (cm)	57.1[b]	71.7[a]	66.1[a,b]
Stem biomass (g)	3.38[a]	2.92[a]	2.94[a]
Root biomass (g)	4.12[a]	2.63[b]	2.53[b]
Stem diameter (mm)	11.0[a]	10.2[b]	9.8[b]
Root volume (ml)	6.2[a]	4.5[b]	4.6[b]
Lateral root area (cm^2)	9.17[a]	9.17[a]	9.04[a]
Tap root length (cm)	17.2[b]	21.4[a]	18.3[b]
Primary lateral roots (#)	33[b]	39[a,b]	46[a]

higher number of first-order lateral roots than did the other seedling types, but there were no significant differences in the area of the primary lateral roots in the three types of seedlings. The total volume of taproot and lateral roots in the bareroot seedlings was greater than that of either container seedling type (table 3).

Bayou Macon Wildlife Management Area, LA

Seedling height, the only continuous variable measured at the end of the first growing season at Bayou Macon WMA, was significantly different among all three seedling treatments. The inoculated container seedlings were 20 percent and 79 percent taller than the noninoculated container and bareroot stock, respectively (table 4). The categorical data analyses did not detect any significant differences in basal sprouting or dieback among seedling treatments. Both types of container seedlings experienced greater herbivory by deer than the bareroot seedlings, but the differences were significant only between the bareroot and non-inoculated container seedlings (table 4). Survival among the inoculated container seedlings was 97 percent, which was significantly higher than that of the noninoculated container and bareroot stock (i.e., 88 percent and 79 percent, respectively) (table 4).

Table 4—Comparison of mean first-year height and survival among seedlings planted at Bayou Macon WMA in NE Louisiana (means within a row with same superscript are not significantly different, $\alpha = 0.05$)

	Variable		
Seedling treatment	Height	Survival rate	Deer browse
	Cm	- - - - -*Percent*- - - - -	
Bareroot	32.8^{c}	78.8^{b}	3.4^{b}
Container	48.8^{b}	87.5^{b}	15.6^{b}
Inoculated container	58.7^{a}	96.6^{a}	$7.3^{a,b}$

DISCUSSION

Morphological differences between the 1+0 bareroot and container-grown seedlings were significant and can be used, in part, to explain the differences in the survival and condition of the seedlings during the first growing season after planting. The root systems of the bareroot stock consisted primarily of a large taproot with roughly half as many primary lateral roots as the container-grown seedlings. This lack of root surface area has implications for reduced water and nutrient uptake, which may have offset the growth potential advantages associated with the larger biomass and height of the bareroot seedlings.

Differences in first-year survival among the three seedling types at Yazoo NWR were difficult to evaluate because most of the container seedlings were clipped at or near the root collar by rodents. Few container-grown seedlings sprouted after they were clipped in mid-August. However, both the bareroot and container-grown seedlings may resprout during the second growing season.

Morphological differences between seedling types diminished greatly after the first growing season at Alazan Bayou WMA, which had the most efficient natural drainage of the three sites. In addition, height growth among seedlings had shifted, favoring the container stock, even though partial dieback was more prevalent among the container stock. There was no significant difference in survival among the seedling types at this site. However, the noninoculated container seedlings grew significantly taller than the bareroot seedlings, despite the greater rate of partial dieback. The high rate of partial dieback among both types of container-grown seedlings could be related to one or more of the following factors: a higher demand for water associated with their more highly developed root systems, increased vulnerability of their more slender stems to damage by ice and freezing temperatures, and, possibly, the indirect effects of biweekly fertilization.

After the first growing season, the number of lateral roots in the bareroot stock was no longer significantly lower than that of the noninoculated container stock, but the number of lateral roots in the inoculated container seedlings was significantly higher than the other two seedling types. The noninoculated container seedlings grew significantly taller than the bareroot stock, but both types of container seedlings were twice as prone to dieback as the bareroot stock.

At Bayou Macon WMA, the drainage of local precipitation from the field was very slow, but it was not inundated by backwater flooding. The significance of the improved survival among the inoculated container stock at Bayou Macon WMA is possibly related to the more highly developed root system of these seedlings, which was induced by confinement in containers, frequent watering and fertilization, and more extensive mycorrhizal development.

CONCLUSIONS

Seedling culture clearly influenced the morphology of the root systems in the three types of seedlings used in these experiments. The highly fibrous root system of the container-grown seedlings may present potential advantages when planted at sites that are prone to drought and/or flooding. Inoculation of the container seedlings with vegetative mycelia of *P. tinctorious* slightly, but significantly, enhanced root fibrosity.

During the first growing season at Alazan Bayou WMA, the morphological differences between seedling types diminished. At this site, which had the most efficient drainage of the three planting sites, there were no significant differences in seedling survival among the three seedling types. The container-grown seedlings were twice as prone to dieback as the bareroot seedlings; however, their overall height growth was greater. Height growth was also greater among the container-grown seedlings at the Louisiana site. At Bayou Macon WMA, where drainage was poor but there was no long-term inundation, significant improvements in first-year survival and growth among the inoculated container stock could be attributed to seedling culture and related differences in root morphology. At Yazoo NWR, loss of stem tissue in 98 percent of the container-grown seedlings to small mammals precluded a meaningful comparison of first-year survival among the three treatments.

Seedling sprouting and survival during the second and third growing seasons should provide further insight into the relative importance of seedling type. The first year after planting, however, appears to be a very critical period in which seedling morphology, flooding, and herbivory have significant impacts on growth and survival, especially on sites that are prone to flooding.

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