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Effect of midstory and understory removal on the establishment and development of natural and artificial pin oak advance reproduction in bottomland forests

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Abstract Regenerating oaks (*Quercus* L.) on mesic and hydric sites has remained a problem largely because of inadequate density and poor distribution of large oak advance reproduction prior to harvesting. We examined the effect of midstory and understory removal on the establishment and 3-year development of natural and artificial sources of pin oak (*Q. palustris* Muenchh.) advance reproduction in bottomland forests in southeastern Missouri, USA. Midstory and understory removals increased the photosynthetically-active radiation (PAR) reaching the seedling layer from about 3 to 15%. This increased light did not increase the density of natural pin oak advance reproduction compared to control, but it increased the survival and nominally increased the growth of the natural pin oak advance reproduction. Where the midstory and understory had been removed, underplanted RPM® container stock and bareroot pin oak stock maintained high survival, but of the two only the RPM® stock maintained positive height and diameter growth while bareroot stock suffered some growth reductions. Pin oaks originating from the direct seeding of stratified acorns sown in the spring had low germination and survival,

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but the survivors had growth rates similar to those of natural seedlings in thinned stands. Applying triclopyr to competitors in the ground flora layer only nominally increased PAR but reduced the percent survival and marginally increased the growth of natural and artificial pin oak. We conclude that artificial reproduction may be used to further increase the probability of achieving adequate numbers of the desired species in the future. Bareroot seedlings may not perform as well as RPM® seedlings and natural seedlings already present. However, bareroot and RPM® seedlings remained significantly larger than the natural seedlings after 3 years.

Keywords Midstory removal · Photosynthetically-active radiation · Natural regeneration · Artificial regeneration · Underplanting

Introduction

Regenerating oaks (*Quercus* L.) on mesic uplands and in hydric bottomlands has long been a matter of concern (Hodges and Gardiner 1993; Sander and Graney 1993; Meadows and Stanturf 1997; Johnson et al. 2002). Unsatisfactory oak regeneration following overstory removal in bottomland stands is often due to inadequate density and poor distribution of oak advance reproduction before the overstory removal treatment (Jenkins and Chambers 1989; Lockhart et al. 2000). Success of oak regeneration is also contingent upon the size of oak advance reproduction to ensure that they are competitive through the regeneration period and able to maintain dominance as they recruit into the overstory (Johnson et al. 2002). Advance reproduction in oak-dominated stands on higher quality sites is often lacking or is too small to effectively compete following regeneration harvesting (Stringer 2005). After regeneration harvesting, smaller oak seedlings cannot compete as effectively as larger advance reproduction of shade tolerant species or faster-growing, shade intolerant competitors. In the southern Appalachians, Loftis (1990a) demonstrated that the probability that northern red oak (*Q. rubra* L.) advance reproduction was dominant following final shelterwood removal increased as initial seedling size increased, an observation that was confirmed by Spetich et al. (2002) who regenerated northern red oak by underplanting in shelterwoods in the Boston Mountains. It has similarly been suggested that larger (>30 cm tall) red oak advance reproduction are more likely to take advantage of increased light levels following overstory removal than are smaller (<30 cm tall) seedlings (Belli et al. 1999).

One major factor contributing to low density of small oak advance reproduction is the amount of light reaching the forest floor in many of these forests (Janzen and Hodges 1985; Gardiner and Helmig 1997). Low light levels (<5% of full sunlight) beneath a closed canopy forest with a dense midstory and understory of shade-tolerant vegetation lower the survival and limit the growth of oak advance reproduction (Hodges and Gardiner 1993; Lorimer et al. 1994; King and Grant 1996; Gardiner and Hodges 1998; Parker and Dey 2008). The development of shade tolerant understories and succession from oak-dominated forests to more mixed mesophytic species compositions has been accelerated by fire suppression and partial cutting practices (Abrams 1998; Nelson et al. 2008; Nowacki and Abrams 2008). Species such as red maple (*Acer rubrum* L.), American elm (*Ulmus americana* L.), and green ash (*Fraxinus pennsylvanica* Marsh.) are common in the midstories and understories of many bottomland forests in this region (Nelson et al. 2008). These competing species are often significantly more shade and flood tolerant than are many of the co-occurring oak species (Bey 1990; Kennedy 1990; Walters and Yawney

1990), and they can effectively limit the growth of oak advance reproduction through competition for light, nutrients, and water (Kolb and Steiner 1990).

Forest structure can be managed by thinning and harvesting to reduce stand density to allow more light to reach the forest floor and possibly benefit oak advance reproduction (Jenkins and Chambers 1989; Lorimer et al. 1994; Miller et al. 2004). Removing the midstory trees by cutting and stump treatment with Tordon 101R® increased height growth in cherrybark oak (*Q. pagoda* Raf.) advance reproduction by 1.5–3 times compared to seedlings in unthinned control stands in bottomland forests after 9 years (Lockhart et al. 2000). Similarly, a shelterwood harvest that reduced basal area by 56%, leaving a residual density of 11 m^2 ha^{-1}, increased height growth of underplanted northern red oak seedlings by 11 times and diameter growth 6 times compared to the control treatment after 3 years in a mixed hardwood forest (Tworkoski et al. 1986). Shelterwood harvesting has been shown to promote development of oak advance reproduction and improve oak dominance probabilities after regeneration release through final shelterwood removal (Loftis 1990b; Schlesinger et al. 1993; Spetich et al. 2002). Higher shelterwood density and woody competition control is recommended on sites of higher quality to control competition from shade tolerant woody species, growth of stump sprouts of less-desirable species, and fast growing shade intolerant regeneration.

Allowing more light to reach the understory may benefit competitors in addition to the desired species. Heavier cuts that provide more sunlight to the forest floor tend to favor fast-growing, shade-intolerant species, while lighter cuts tend to favor less-desirable, shade-tolerant species (Clatterbuck and Meadows 1993; Meadows and Stanturf 1997). If oak advance reproduction is not present at the time of overstory removal, competing species are likely to benefit and eventually outgrow oak seedlings that establish later (Stringer 2005). For this reason, it is important that treatments encourage large oak advance reproduction to develop while simultaneously minimizing the establishment and growth of competitors (Loftis 1990b; Schlesinger et al. 1993).

Building adequate numbers of large oak advance reproduction needed to achieve oak stocking in the future stand can be done through natural regeneration processes, or by artificial methods such as direct seeding of acorns, or by planting bareroot or container oak seedlings (Johnson 1984; Loftis 1990b; Wittwer 1991; Spetich et al. 2002). The benefits of establishing oak by direct seeding, or planting bareroot and large container seedlings in flood plains has been reviewed by Allen et al. (2004) and Dey et al. (2008). Direct seeding acorns was popular in the 1980s, largely in the Lower Mississippi Alluvial Valley, due to the reduced cost of establishment and the increased portion of the year that sowing could be done compared to tree planting. Today, managers prefer tree planting over direct seeding for regenerating oaks because of better survival and growth in the long-term (Allen et al. 2004; Dey et al. 2008).

Planting seedlings beneath a shelterwood is a recommended method for artificial reproduction (Loftis 1990b; Johnson et al. 2002; Gardiner and Yeiser 2006). Dey and Parker (1997) found that after 2 years, northern red oak seedlings planted under a shelterwood treatment in central Ontario, Canada, exhibited 55% larger diameters and were twice as tall as seedlings planted under an uncut overstory. They concluded that shelterwoods that provide adequate light at the forest floor promote the establishment and enhance the performance of underplanted seedlings. However, the number of studies on regenerating oak using midstory thinning or shelterwood harvesting is limited in number of different species evaluated and have been done largely in upland forests.

This study was designed to evaluate the effects of midstory and understory removal and ground flora competition control on the establishment and development of natural pin oak reproduction and artificial pin oak reproduction established by planting and seeding in bottomland forests within the Lower Mississippi Alluvial Valley. The first objective of this study was to quantify the effects of midstory and understory removal on the (1) quantity of photosynthetically-active radiation (PAR, 400–700 nm) reaching the tree seedling layer, (2) the establishment, survival, and growth of natural pin oak advance reproduction and (3) the survival and growth of artificial pin oak reproduction.

Methods

Study sites

This study was conducted in two mature (80+ years old) bottomland hardwood forest stands located in adjacent greentree reservoir management pools in Stoddard County, southeastern Missouri, USA (N 37°01′00.00″ W 090°06′03.50″). One pool was located in Mingo National Wildlife Refuge managed by the U.S. Fish and Wildlife Service (US-FWS). The other pool was located in Duck Creek Conservation Area managed by the Missouri Department of Conservation (MDC).

Keys et al. (1995) identified this area as Ecological Subsection 234Ac, the White and Black Rivers Alluvial Plain having annual precipitation averaging 127 cm, annual climate averaging 14°C, and annual growing season averaging 205 days. Nigh and Schroeder (2002) further identified the area as being of the MB1d Mingo Silty Lowland land type association. Soils at these sites were mapped as Calhoun Silt Loam (fine-silty, mixed, active, thermic Typic Glossaqualfs) with an approximate pin oak site index of 24 m (index age 50) (Butler 1985). This soil typically develops in nearly level, poorly-drained terraces and floodplains. Characteristics of this soil include high available water capacity, low fertility, low organic matter, and slow permeability and runoff.

At the study sites, pin oak was the dominant species (54% of the basal area). Other important overstory and midstory species included sweetgum (*Liquidambar styraciflua* L.), overcup oak (*Q. lyrata* Walt.), red maple, American elm, willow oak (*Q. phellos* L.), green ash, persimmon (*Diospyros virginiana* L.), and cherrybark oak (Krekeler et al. 2006). The regeneration layer was dominated by seedlings of red maple, green ash, sweetgum, American elm, pin oak, and willow oak. Trumpet creeper (*Campsis radicans* L.) was also abundant in many parts of the study area, often completely overtopping the seedlings present on the forest floor.

Much of this area was logged and drained in the 1800s for agriculture (Robertson et al. 1984). Due to the hydrology of the area, however, it was difficult to keep the area drained and thus it was subsequently abandoned as an agricultural area. The heavy logging, repeated fires, land clearing, and partial drainage of the area through the late 1800s and early 1900s did, however, change the forest composition from what historically was a cypress-tupelo swamp forest to the present oak-dominated bottomland forest (Thompson 1980). Mingo National Wildlife Refuge was established by the USFWS in 1945 and Duck Creek Conservation Area was established by the MDC in 1950 and both areas have been managed for waterfowl habitat and hunting since acquisition (Merz and Brakhage 1964) and are flooded nearly annually during the fall waterfowl migration and hunting season. Before 1999, the management pools were flooded to depths of 15–50 cm before waterfowl hunting season and drained after the season ended. Since that time, managers have varied the timing and duration of flooding to more closely resemble the natural hydrologic cycle and seasonal weather conditions by flooding some of the pools later for shorter durations during dry years and earlier for longer durations during wet years.

Design

A randomized complete block design was used, with six blocks, three located in Mingo National Wildlife Refuge and three at Duck Creek Conservation Area. Each block was approximately 4 ha and contained nine 0.45-ha treatment units. The blocks were positioned and configured so that they were internally homogeneous in stand conditions. Blocks were located on elevations where pin oaks were likely to be managed. In the center of each of the nine treatment units, a circular, 0.08-ha plot was used for inventory and classification of all trees $\geq$4 cm dbh. All artificial reproduction was also established in the 0.08-ha plot area to minimize any potential edge effects. Within these 0.08-ha plots, five 0.004-ha subplots were located, one in the center of the 0.08-ha plot and one at a distance of 15 m from the center in each cardinal direction. These were used for the inventory of trees $\geq$1.37 m tall but <4 cm dbh. Within each 0.004-ha subplot, a 0.0004-ha subplot was established for the inventory of trees <1.37 m tall.

Treatments

One of nine treatments was randomly assigned to each treatment unit within each block. The nine treatments included midstory and understory removal (designated as “removal’) in combination with each of four pin oak seedling types (natural, direct seeded, 1-0 bareroot, and “Root Production Method®” or “RPM®” container seedlings) with and without a ground flora competition control treatment (designated as “gfc”), plus one control (Table 1). The purpose of the midstory and understory removal treatment was to increase the amount of photosynthetically-active radiation (PAR) reaching the level of the oak seedlings. The different artificial reproduction methods were selected to provide a reasonable comparison to the alternative of relying on natural reproduction. The ground flora control treatment was conducted to remove competing herbaceous vegetation, as well as less-desirable seedlings and woody vines that would potentially be released by the midstory and understory removal treatment. These treatment combinations afforded the opportunity to examine the effect of the removal-only and removal + gfc treatments on the accumulation, survival, and growth of natural pin oak reproduction in unaltered forest and the survival and growth of artificial stock compared to natural seedlings in the treated stands.

In the removal treatment, all midstory and understory non-oaks 1 cm dbh or larger that were below the main canopy were deadened with herbicide. We applied 1 ml of imazapyr (20% concentration of active ingredient using Arsenal® AC) into hacks made in the tree bole with a hatchet having a 3-cm bit. A single hack plus 1 ml of herbicide was applied 1.37 m above ground for every 7.5 cm in dbh. The removal treatment was applied during February 2003 across each entire 4-ha block except in the control treatment at a cost of approximately \$250 ha^{-1}. The majority of the treated trees were red maple, green ash, American elm and sweetgum, all of which were found in the greatest abundances in midstories of these forests. All treated trees were revisited after the first growing season, and those that had not died were re-treated with herbicide.

In April of 2003, pin oak acorns were planted within the 0.08-ha plots in all experimental units designated for direct seeding. The acorns were purchased from the George O. White State Nursery in Licking, Missouri and were of undetermined origin. They had

Table 1 Treatment combinations compared in the study

Stock	Control	Midstory and understory removal	
		With ground flora competition treatment (Removal + gfc)	Without ground flora competition treatment (Removal only)
Natural reproduction	X	X	X
Direct-seed		X	X
1-0 Bareroot		X	X
RPM® container		X	X

Midstory and understory thinning treatments were applied to all non-oaks as small as 1 cm dbh. Ground flora control treatment was a foliar application of triclopyr to all woody and herbaceous vegetation surrounding each tapped pin oak seedling. The control treatment was not thinned and only natural pin oak reproduction was monitored. Stock types (all pin oak) included natural seedlings ≤1 year old, seedlings from direct-seeded acorns, 1-0 bareroot seedlings, and 1-year-old RPM® container (11-l) seedlings

been collected during the preceding autumn and screened for soundness by float testing, stratified, and stored according to standard nursery practices. In each 0.08-ha plot, 40 acorns were planted by hand 7.5 cm deep and approximately 5 m apart in concentric circles around the plot center. This depth was selected to minimize predation by rodents, waterfowl, and other animals. All planting locations were marked with a wire flag for future reference.

Also in April of 2003, 22 1-0 bareroot pin oak seedlings grown at the George O. White State Nursery in Licking, Missouri, and 22 RPM® container pin oak seedlings (in 11-l pots) grown at the Forrest Keeling Nursery in Elsberry, Missouri, were planted in their respective treatment units. We evaluated the RPM® container seedlings because the nursery procedure produces pin oak seedlings having basal diameters more than 2.5 times greater and root volumes of more than eight times greater than typical bareroot stock available in this region (See Dey et al. 2004; Shaw et al. 2003 for more information about RPM® container seedlings). Seed for the production of the RPM® seedlings was collected the previous fall from Duck Creek Conservation Area. Each of the seedlings was marked with a numbered aluminum tag and planted approximately 6 m apart in concentric circles around the plot center. In treatment units designated for natural reproduction, ten natural pin oak seedlings within the 0.08-m plots were selected and marked with numbered tags. Those individuals that were 1 year old (as evidenced by presence of an attached acorn) were favored, but in areas where there were not sufficient numbers of 1-year-old seedlings, larger seedlings that were representative of the plot were selected.

In June of 2003, the ground flora control treatment was applied within each 0.08-ha plot of those units designated for this treatment. For the ground flora control, Garlon® 3A (20% concentration) was applied with a Solo® backpack sprayer to the foliage of all woody and herbaceous vegetation surrounding each tagged pin oak seedling (both natural and artificial reproduction). Tagged seedlings were shielded during the herbicide application in an attempt to minimize injury caused by exposure to the herbicide.

Measurements

The initial basal diameters (measured 2.5 cm above the root collar) and heights of all tagged seedlings were recorded at the time of planting. All live seedlings were re-measured following the first (2003), second (2004), and third (2005) growing seasons. During each measurement period, we examined seedlings for browse or other signs of damage but found little or none. Canopy density was measured at each seedling using a spherical crown densiometer (Lemmon 1956) during the middle of each growing season. Measurement of dbh, crown position (open-grown, dominant, codominant, intermediate, overtopped, broken/fallen), dieback class (based on percent of canopy dieback), and survival of all trees ≥4 cm dbh in the circular 0.08-ha plots was conducted before treatment initiation during the summer of 2002, and again during the summer of 2005. Counts by species were also made of all natural reproduction <1.37 m tall in the five 0.0004-ha subplots before treatment, and during the first and third growing seasons after treatment. Counts by species were also made for all trees ≥1.37 m tall and <4 cm dbh in the five 0.004-ha subplots during the third growing season.

During the third growing season, PAR Smart Sensors (Onset Computer Corp., Bourne, MA, USA) were used to determine the amount of PAR penetrating the canopy and reaching the seedling level. These sensors were mounted on steel U-posts, with specially designed mounting brackets, and leveled at a height of 0.9 m, approximately the height at or just above the seedlings. Each sensor was connected to a HOBO® Weather Station logger (Onset Computer Corp., Bourne, MA, USA) for continuous data logging. Four sensors were used simultaneously in four different locations. One sensor was always in an open area receiving full sunlight. The other three sensors were positioned respectively in the centers of the control treatment, one replicate of the removal-only treatment and one replicate of the removal + gfc treatment in each block. These sensors remained in place for approximately 48 h so that PAR data could be logged continuously for an entire day before they were moved to another block. We logged the PAR readings for the entire day in an effort to reduce the variation associated with instantaneous readings caused by the movement of sunflecks throughout the day. The temporal variation in light levels has been demonstrated to far exceed the spatial variation in closed-canopy stands (Messier and Puttonen 1995).

Also during the third growing season, a LI-6400 portable infrared gas analyzer (Li-Cor Biosciences, Inc., Lincoln, NE, USA) was used to determine the photosynthetic response of pin oak seedlings under optimal conditions. We conducted these measurements in the removal-only treatment on the natural, bareroot, and RPM® seedlings, and on natural seedlings in the control. The direct-seeded acorn treatment was not examined due to extremely poor (4%) survival, which limited the sample size. Ground flora control treatments were also not selected to eliminate any influence that the herbicide may have had on the seedlings. Measurements were made within the designated treatment units for each block on 14 and 15 June 2005 and were repeated on the same individuals on 2 August 2005. Four apparently healthy seedlings within each treatment were selected, and measurements were made on one leaf per seedling between 0900 and 1600 h. Relative humidity was maintained at ambient values while temperature (30–35°C), CO_2 concentrations (400 μmol mol^{-1}), and irradiance (2,000 μmol m^{-2} s^{-1}) were kept constant. Following analysis with the LI-6400, each sample leaf was removed, bagged, labeled, and stored in a cooler. Within several days, leaf area was determined by passing each leaf through a LI-3100C leaf area meter (Li-Cor Biosciences, Inc., Lincoln, NE, USA). Following a period of storage in a refrigerator, the leaves were rehydrated for 24 h and the weight of each leaf to the nearest 0.001 g was measured. Specific leaf area (SLA) was calculated by dividing leaf area by leaf mass. Values for photosynthetic response and SLA were averaged for the two sampling periods before analyses.

Because it was impossible to assure that hydrologic conditions would be uniform among treatment units within blocks, soil water content was monitored. To do this, Watermark Sensors (Irrometer Company, Inc., Riverside, CA, USA) were buried 10 cm below the soil surface in the center of each treatment unit. Meter readings were taken weekly during the first growing season from 18 June through 17 September 2003. A laboratory calibration study was conducted with soils from each block to determine the relationship between the meter readings and gravimetric water content. This calibration study allowed for the development of equations for converting meter readings made in the field to estimated gravimetric soil water content. Because we found no significant differences among blocks or treatments within blocks, we were reasonably assured that hydrology was not confounding measures of seedling physiology.

Analysis

We used mixed linear models (PROC MIXED, SAS version 9.1, SAS Institute, Inc. Cary, NC, USA) examine treatment effects. Treatments were evaluated as fixed effects tested with the treatment × block interaction as the random effect in the error term. For repeated measures comparisons, we included a repeated statement (subject = treatment × block) and evaluated compound symmetry and autoregressive covariance structures, generally selecting the covariance structure yielding the lowest AAIC score. For comparing the survival and growth of seedling stock types, we excluded the control treatment from the analysis to eliminate the potentially confounding effect of a full canopy on these parameters. For significant effects ($\alpha = 0.05$), differences among individual (least square) means were determined using Fisher's least significant difference.

Results

Density and light

Removing the midstory and understory had no measurable effect on the residual basal area ($P = 0.22$; Table 2) but it decreased the tree density by nearly 900 trees per ha ($P < 0.01$) and decreased the canopy density by 7–9% ($P < 0.01$) compared to the control. This small reduction in canopy density increased the PAR in the tree seedling layer from about 3% to about 15% of full sunlight ($P < 0.01$). PAR levels were not significantly different between the removal-only and the removal + gfc treatments ($P = 0.72$).

Natural reproduction

The density of natural pin oak seedlings (<1.37 m tall) increased after implementing treatments because of a large acorn crop (Table 3). However, more than half of the pin oak seedlings that developed did not survive two growing seasons. The density of natural pin oak seedlings differed considerably from year to year. However, for any single year, there were no significant differences among the treatments ($P > 0.38$).

For the natural pin oak reproduction that we tagged and monitored, we found that first-year survival for all treatments exceeded 60% but decreased significantly ($P < 0.04$) each year thereafter (Fig. 1). Overall survival rates were about 20–30% greater in the removal-only treatment than in the removal + gfc treatment and in the control. These differences

Table 2 Stand and canopy density and photosynthetically active radiation (PAR) by treatment after the third growing season

	Trees per ha	Basal area ($m^2\ ha^{-1}$)	Canopy density (%)	PAR (% full sunlight)
Control	1,204a	26a	98a	3.3a
Removal only	315b	23a	90b	15.9b
Removal + gfc	318b	26a	91b	16.7b

Treatments are midstory and understory removal with (Removal + gfc) and without ground flora competition (Removal only) treatment compared to control (i.e., no treatment). Data were collected three growing seasons following treatment application. Within columns, means followed by a differing letter are significantly different ($\alpha = 0.05$)

Table 3 Natural pin oak reproduction density (trees < 1.37 m tall) by treatment

	Trees per hectare		
	Pretreatment	Year 1	Year 3
Control	232	6,256	998
Removal only	410	4,774	1,852
Removal + gfc treatment	464	6,175	1,430

Treatments are midstory and understory removal with (Removal + gfc) and without ground flora competition (Removal only) treatment compared to control (i.e., no treatment). Data were collected three growing seasons following treatment application. There were no significant differences ($P > 0.38$) among treatment units prior to or subsequent to treatment

initially were statistically significant ($P = 0.01$) but were not by the end of the third growing season ($P > 0.13$). Of the survivors, there was a nominal but not statistically significant decrease in the average height ($P > 0.34$) and diameter ($P > 0.24$) during the first year of the study (Fig. 2a, b). The removal and the removal + gfc treatments nominally increased the height and basal diameter growth of surviving natural pin oak reproduction relative to seedlings in the control; however, the differences among treatments were not statistically significant ($P > 0.25$).

Artificial reproduction versus natural reproduction in treated stands

The first-year survival of bareroot, RPM® and natural reproduction in the removal-only and the removal + gfc treatments (combined) exceeded 80% (Fig. 3a). This was about the same ($P > 0.28$) as for the natural reproduction in the removal only and the removal + gfc treatments (combined). The germination of direct-seeded acorns was less than 9%, with only 26 seedlings present at the end of the first growing season. During the subsequent 2 years, the survival of the bareroot and RPM® stock remained about the same ($P > 0.51$), and after the third growing season the survival of each of these stock types was nearly two times greater ($P < 0.01$) than the survival of the natural reproduction in the treated plots. The survival of the direct-seeded stock decreased nominally to about 4% and remained significantly lower ($P < 0.01$) than the other stock types.

Compared to the removal-only treatment, the removal + gfc treatment decreased the survival of all pin oak reproduction types by 10–20% points ($P > 0.06$; Fig. 3b) and

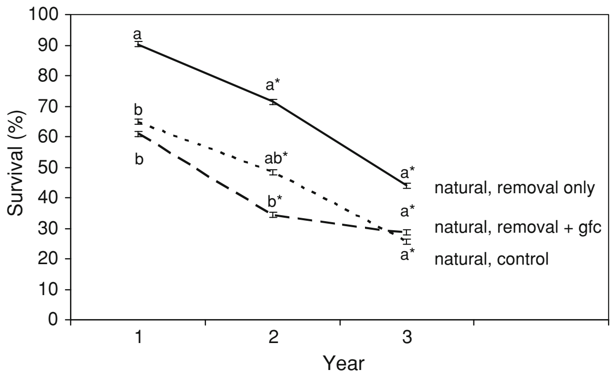

Fig. 1 Percent survival of pin oak natural reproduction by treatment determined at the end of each growing season. Treatments included midstory and understory removal (removal only), removal plus ground flora control of competitors (removal + gfc), compared to control. For each year, different letters indicate significant differences among treatments. Bars are ±one standard error. For each treatment, an asterisk indicates a significant change in survival from the previous year. For all comparisons, $\alpha = 0.05$

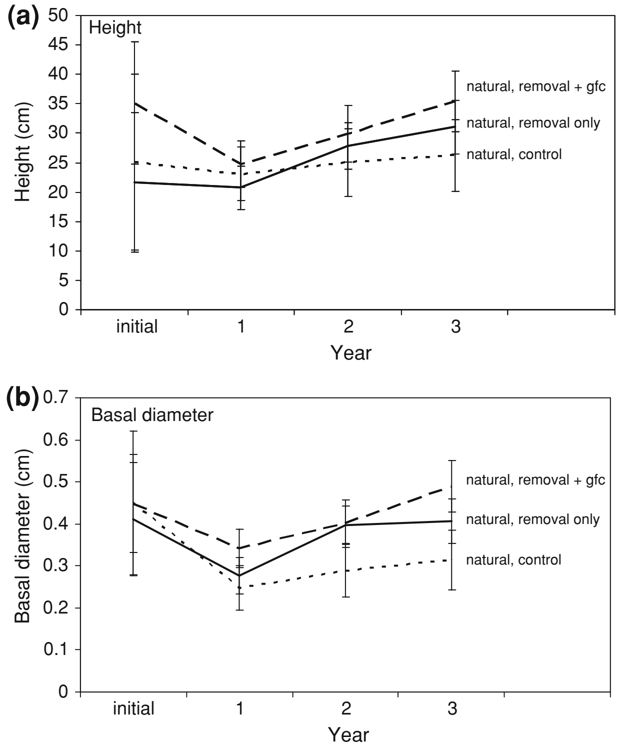

Fig. 2 Height (**a**) and basal diameter (**b**) of pin oak natural reproduction by treatment. Treatments included midstory and understory removal (removal only), removal plus ground flora control of competitors (removal + gfc), compared to control. Bars are ±one standard error. There were no significant differences among treatments ($\alpha = 0.05$)

appeared to affect all stock types in the same manner. However, controlling competitors in the ground flora layer did nominally increase the height and diameter growth of all stock types of pin oak seedlings but these growth increases were small in magnitude (about 6–7%) and not statistically significant ($P = 0.60$). Consequently, we were able to combine the data from the removal-only and removal + gfc treatments to examine stock type growth over time.

At the time of planting, the RPM® stock averaged 88 cm tall, nearly 1.5 times larger than that of the bareroot stock ($P = 0.01$) and more than three times larger than the natural advance pin oak reproduction ($P = 0.01$; Fig. 4a, b). All of the artificial stock types exhibited significant ($P = 0.01$) height growth during the first year but the RPM® seedlings were the only stock to maintain statistically significant height growth ($P = 0.04$) during subsequent years. The height of the direct-seeded stock also increased and its height trend was similar to that of the natural pin oak reproduction. Bareroot seedlings actually suffered height losses ($P = 0.01$) by the end of the second year due to stem dieback. Diameter growth generally followed a trend similar to that of height growth.

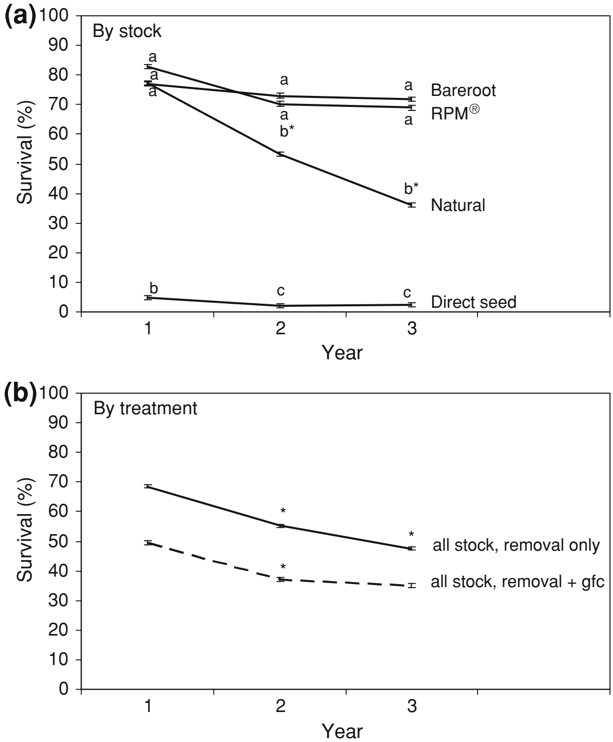

Fig. 3 Percent survival of pin oak artificial stock compared to natural seedlings in removal and removal + gfc treatments combined (**a**) and for all stock combined by treatment (**b**). Stock types included 11-l container seedlings grown by the Root Production Method® (RPM®), 1-0 bareroot seedlings, seedlings from direct seeding, and natural advance reproduction tagged prior to implementing treatments. Bars are ±one standard error. For each year, different letters indicate significant differences among stock types or treatments. For each stock type or treatment, an asterisk indicates a significant change in survival from the previous year. For all comparisons, $\alpha = 0.05$

Natural reproduction in the removal treatment had greater light-saturated photosynthesis rates (A_{max}) ($P = 0.01$) than did natural reproduction in the control and was statistically the same ($P = 0.25$) as the RPM® stock (Table 4). Bareroot seedlings had nominally, but not significantly greater A_{max} values ($P = 0.12$) than natural pin oak reproduction in the control. We found no statistical differences in SLA among stock types or treatments ($P = 0.41$).

Discussion

Stand density and light

The midstory and understory was removed to increase the amount of PAR reaching the forest floor, and ultimately to increase the density of advance pin oak reproduction and to allow the existing pin oak advance reproduction to become larger and presumably more competitive while not releasing existing competing vegetation. In undisturbed controls we observed PAR levels of about 3% of full sunlight (Table 2) which is consistent with reports of light levels of less than 10% in undisturbed mature bottomland hardwood stands in

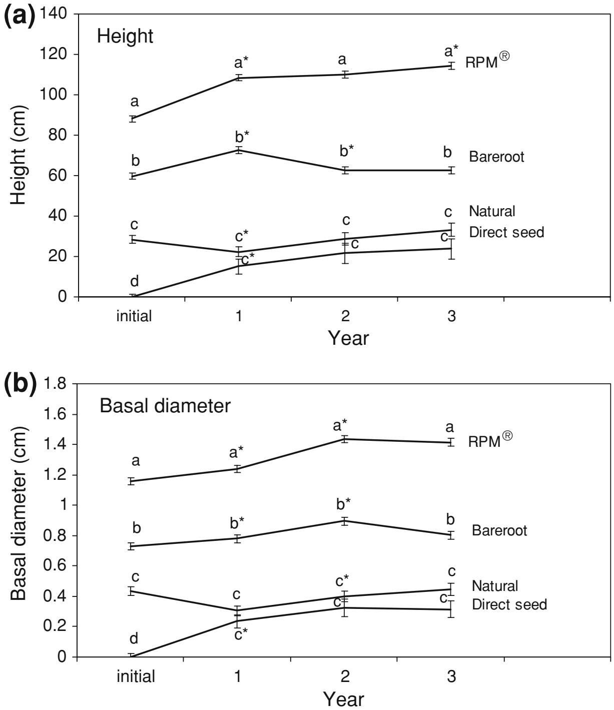

Fig. 4 Height (**a**) and basal diameter (**b**) of artificial pin oak reproduction compared to natural reproduction by stock type. Stock types included 11-1 container seedlings grown by the Root Production Method® (RPM®), 1-0 bareroot seedlings, seedlings from direct seeding, and natural advance reproduction tagged prior to implementing treatments. Treatments included midstory and understory removal (removal only), removal plus ground flora control of competitors (removal + gfc) combined. Bars are ± one standard error. For each year, different letters indicate significant differences among stock types. For each stock type, an asterisk indicates a significant change from the previous year. For all comparisons, $\alpha = 0.05$

Table 4 Average net light-saturated photosynthesis (A_{max}) and specific leaf area for natural and artificial pin oak stock in the control treatment and where the midstory and understory was removed

Treatment	Light-saturated photosynthesis (A_{max}) (μmol m^{-2} s^{-1})	Specific leaf area (cm^2 g^{-1})
Natural reproduction (control)	3.9a	101a
Natural reproduction (removal only)	7.9b	89a
Bareroot (removal only)	5.9ab	92a
RPM (removal only)	6.8b	95a

Light-saturated photosynthesis was determined under ambient relative humidity at constant temperature (30–35°C), CO_2 concentration (400 μmol mol^{-1}), and irradiance (2000 μmol m^{-2} s^{-1})

Within columns, means followed by a differing letter are significantly different ($\alpha = 0.05$)

Louisiana (Jenkins and Chambers 1989), of less than 4% in bottomland forests in Georgia (Ostrom and Loewenstein 2006) and in mesophytic upland forests in West Virginia (Miller et al. 2004), and of less than 1% in northern hardwood forests of Ontario (Dey and Parker 1997). Removing the midstory and understory vegetation increased the PAR five fold to about 15% in our study (Table 2), which is comparable to the findings of Lockhart et al. (2000) who observed a four-fold increase in PAR and to Ostrom and Loewenstein (2006) who observed nearly a three-fold increase in PAR following midstory removal treatments in bottomland forests in the southeast USA. Although we measured PAR during the third growing season following midstory and understory removal, Miller et al. (2004) showed that midstory and understory removals can enhance light levels reaching the understory for several years.

Controlling the competition of the non-oak seedlings and woody vines in the ground flora nominally increased the PAR compared to the removal-only treatment (Table 2). The removal + gfc treatment was examined because of the concern that the removal-only treatment would cause competing seedlings and woody vines to grow larger and compete more aggressively than oak advance reproduction. However, we found little evidence that this would happen during the first 3 years following the application of the removal-only treatment, suggesting that the increased light levels reaching the forest floor were insufficient to release competing vegetation.

The removal-only and removal + gfc treatments used in our study were similar to subcanopy treatments evaluated for regenerating oaks in mesophytic uplands in the southern Appalachians (Loftis 1990b) and in bottomlands in southern North America (Janzen and Hodges 1987). The authors reported that these treatments failed to increase the establishment of new oak seedlings (Janzen and Hodges 1987; Loftis 1990b). Collins and Battaglia (2008) found that the establishment and early survival and growth of oaks in bottomlands were greater under a full canopy compared to under experimentally-created canopy gaps. We found no statistical differences in the density of pin oak advance reproduction that became established in either of the removal treatments compared to control suggesting that these treatments offered little or no establishment advantage compared to leaving the forest untreated. However, the removal or removal + gfc treatments did not inhibit the establishment of pin oak reproduction and we observed a ten- to twenty-fold increase in the pin oak seedling density 1 year after initiating the treatments largely resulting from a good acorn crop (Table 3). Fewer than half of the seedlings that became established soon after we began the experiment did not persist for more than 2 years regardless of treatment.

Although the removal-only and removal + gfc treatments did not increase the density of pin oak advance reproduction in this study, they reportedly allow the advance reproduction present on the site to grow larger and thereby become more competitive with other tree species when eventually related by harvesting the residual overstory (Janzen and Hodges 1987; Loftis 1990b; Lorimer et al. 1994). In addition, inadequate natural reproduction can also be supplemented with artificial oak reproduction before regenerating stands (Clatterbuck and Meadows 1993). It is with these considerations in mind that we compared the survival and growth of the natural pin oak reproduction present before implementing treatments to that of the artificial pin oak reproduction established by direct seeding, and by planting 1-0 bareroot seedlings and 11-l RPM® container seedlings.

Natural reproduction

We found that the removal-only and removal + gfc treatments increased the first-year percent survival of the natural pin oak reproduction (Fig. 1). The percent survival remained nominally higher than for the removal + gfc and the unthinned control treatments throughout the monitoring period, indicating that drift or flashback from the triclopyr applied to the non-oaks in the removal + gfc treatment may have reduced the survival of the natural pin oak reproduction. Others have documented that triclopyr applications generally have caused little or no injury to non-target vegetation when applied to cut stumps or injected into the boles of trees (Kochenderfer et al. 2001; Walter et al. 2004). The foliar application of triclopyr to competitors in the ground flora layer only nominally increased the growth of surviving pin oaks (Fig. 2a, b) but these growth increases were statistically insignificant and biologically unimportant, suggesting that the addition of the gfc treatment was unnecessary. In fact several studies have highlighted potential unintended consequences when using herbicides to control ground flora competition. For example, controlling competition with glyphosate in bottomland forests reduced oak seedling height growth compared to control treatments; however it was suggested that other environmental factors may have contributed to the growth reduction where the competition was controlled Wittwer (1991). Similarly, glyphosate sprayed on the cut stumps of less-desirable species during the application of a midstory and understory thinning, resulted in the mortality of a number of smaller desirable seedlings (Janzen and Hodges 1987) and direct-sprayed glyphosate herbicide treatment applied to competing vegetation around planted cherrybark oak seedlings reduced their height growth and failed to increase their diameter growth or survival in a South Carolina bottomland forest during a 7-year period (Nix and Cox 1987). Following a study that examined competition control by single-spot application glyphosate after planting bareroot Nuttall oak (*Q. nuttallii* Palm.) seedlings in a bottomland site in west-central Mississippi, Ware and Gardiner (2004) indicated that herbicide application in hardwood stands can potentially favor desirable species, but improper application has the potential to damage desirable stems as well. Our findings show that the growth benefits of controlling the competition with triclopyr were minor at best and probably do not justify the expense of application or the risk to reproduction of desirable species.

Artificial reproduction versus natural reproduction in treated stands

We found that the survival of the bareroot and RPM® container stock remained above 70% and was considerably greater than that of the natural reproduction (Fig. 3a). High survival rates for underplanted oak seedlings in shelterwoods have been reported, often exceeding

85–99% after 2–5 years (Dey and Parker 1997; Hodges and Janzen 1987; Pope 1993; Spetich et al. 2002). The survival of underplanted stock has been shown to be greater when the planted seedlings have a large number (>7) of first-order lateral roots (Kormanik et al. 1995). Although not as high as reported elsewhere, the survival of the bareroot and RPM® stock in our study was nearly double that of the natural reproduction by the end of the third growing season, which we attribute to the larger diameters and shoot lengths of these two artificial stock types.

The low germination and survival rates (4% after 3 years) of the direct-seeded acorns were quite unexpected, and undoubtedly a combination of factors contributed to the poor success. Wittwer (1991) reported satisfactory survival of 45% for direct-seeded Shumard oak (*Q. shumardii* Buckl.) and willow oak after 1 year, with a decline to 35% after 3 years on a southeastern Oklahoma harvested bottomland site prepared by shearing, windrowing, and burning of the windrows. Johnson and Krinard (1985) similarly concluded that 35% survival is a reasonable expectation for direct-seeded red oak species acorns planted on bottomland sites in west-central Mississippi. The acorns used in this study were provided by the Missouri State Nursery and had been collected during the preceding autumn, screened for soundness, stratified, and stored in the same manner as all other red oak group acorns routinely handled by this facility. The acorns were planted within 24 h of being received from the nursery in the spring, so it is assumed that the acorns did not become too dry during handling. Seeding in the spring was intentional due to concerns that acorns sown in the fall would not only be subjected to extensive flooding, but also to predation by rodents, waterfowl, and other animals throughout the fall and winter. Although we did not observe any direct evidence of animal seed predation observed, this may have been a factor as spring-sown acorns may be exposed to greater predation pressure than of those sown in the fall (Sluder et al. 1961). Another factor contributing to the poor germination rates may have been that the acorns in this study were sown at a depth of 7–8 cm in this study and other studies have shown that shallower sowing depths have been effective. Lhotka and Zaczek (2003) found that scarifying the soil in bottomland oak stands with a disk buried acorns to a depth of no more than 5 cm increased acorn germination compared to untreated stands, which they attributed to a combination of the protection of acorns from predation and the reduction in competition for growing space because of the mechanical removal of herbaceous vegetation, vines, and woody competitors. Johnson (1981) found that Nuttall oak acorns sown at a depth of 2.5 cm had significantly higher germination rates than did acorns sown at depths of 5 or 10 cm, concluding that deeper sowing reduces the speed and quality of germination and fails to offer greater protection from predation.

As we found with natural reproduction, the application of triclopyr reduced survival of all stock types by about 20% (Fig. 3b) and resulted in only a marginal and not statistically significantly greater growth. Most of the growth differences occurred among the different stock types (Fig. 4). The RPM® container seedlings were the largest stock type planted and maintained positive height and diameter growth throughout the study, which we attribute to their large and fibrous root system. Shaw et al. (2003) showed that root volumes of year-old pin oak RPM® seedlings were approximately seven times greater than those of 1-0 bareroot stock. This larger root volume provides a greater reserve of stored carbohydrate that can be used during periods of stress. Although the bareroot and RPM® stock maintained similarly high survival, bareroot stock suffered dieback throughout the study period which is commonly attributed to delayed onset of root growth or to root loss prior to planting because of the lifting and handing at the nursery (Johnson et al. 2002). The bareroot stock also had the lowest light saturated photosynthesis values (A_{max}) compared to the RPM® stock and to natural seedlings in treated stands indicating a lower capacity to photosynthesize and generate carbohydrate. Although Parker and Dey (2008) showed that bareroot northern red oak seedlings can quickly acclimate and exhibit physiological function similar to natural seedlings under shelterwoods in central Ontario, we suspect that the slightly lower A_{max} values for the bareroot stock in our study reflect the cumulative effects of the initial root and shoot loss suffered after planting (Table 4).

Although few in number, the direct-seeded stock also maintained positive growth similar to that of natural reproduction in treated stands. The large growth increment observed in direct seeded seedlings is due to the fact that they have a greater growth potential than do existing seedlings. Following germination, oak seedlings rely on carbohydrate reserves stored in the cotyledons and are not greatly affected by the surrounding environment until after they have depleted this carbohydrate store. Despite the relatively large growth increment observed in both the direct-seeded seedlings and the natural seedlings, they remained relatively small compared to the bareroot and RPM® seedlings. There clearly are other advantages of underplanting tall seedlings. The crowns of tall seedlings can remain above some of the competing ground flora and are less likely to be submerged during minor floods. Although not occurring in our study, browsing by deer and other animals can greatly reduce the survival or growth of oak seedlings (Dey et al. 2008). The foliage of tall seedlings is less likely to be browsed. Thus, large seedlings usually have a greater survival rate than smaller seedlings or recent germinants.

PAR reaching the seedling level following the removal treatment was likely lower than required by pin oak for maximum growth. Although we are not aware of specific threshold PAR levels required for optimal growth of pin oak, other seedlings in the red oak group (*Quercus* section *Lobatae*) require 20–50% full sunlight for maximum growth (Hodges and Gardiner 1993; Ashton and Berlyn 1994; Gottschalk 1994). However, achieving maximum growth was not the goal of this study and of other studies using midstory and understory removal to develop large oak advance reproduction. Rather, the goal was to increase PAR sufficiently to allow the oak advance reproduction to become larger and more competitive while simultaneously not releasing the non-oak reproduction, woody vines, and other oak competitors. Lhotka and Loewenstein (2008) showed that underplanted seedlings of yellow poplar (*Liriodendron tulipifera* L.), a species considered to be more shade intolerant than most species of oaks, could maintain early height growth under relatively low light levels ranging from 3 to 21% of full sun. Even though PAR was only slightly increased by the removal treatment, light-saturated photosynthesis (A_{max}) was significantly greater for the natural reproduction in removal treatment than in the control indicating that small increases in the amount of light available to seedlings may result in considerable benefits to pin oak advance reproduction, even if a maximum growth response was not reached. The absence of significant differences in A_{max} or SLA among the planted bareroot and RPM® container artificial reproduction seedlings and the natural advance reproduction in the removal treatment suggested that, at a physiological level, these seedlings were performing similarly. However, three growing seasons may have been too short a time frame for seedlings to fully respond to the light environment created by the removal treatment and to express significant differences among stock types.

Conclusions

In bottomland forests in southeastern Missouri, midstory and understory removals increased the PAR reaching the seedling layer from about 3 to 15%. This increased light did not increase the density of natural pin oak advance reproduction compared to control, but increased the survival and nominally increased the growth of the natural pin oak advance reproduction. Where the midstory and understory had been removed, underplanted RPM® and bareroot pin oak stock maintained high survival, but of the two only the RPM® stock maintained positive height and diameter growth while bareroot stock suffered growth reductions. Pin oaks originating from the direct seeding of stratified acorns in the spring had low germination and survival, but the survivors had growth rates similar to that of natural seedlings in thinned stands. Applying triclopyr to competitors in the ground flora layer only nominally increased PAR but reduced the percent survival and only marginally increased the growth of natural and artificial pin oak reproduction suggesting that the benefits of controlling the competition with triclopyr were minor at best and probably do not justify the expense of application. Artificial reproduction showed great promise to further increase the probability of achieving adequate numbers of the desired species in the future. Bareroot and RPM® seedlings remained significantly larger than the natural seedlings after 3 years, but it appears that bareroot seedlings may not perform as well as RPM® seedlings and natural seedlings already present. In the future it will be necessary to reduce overstory density further to increase light levels to above 15% of full sunlight in order to sustain or increase the growth of pin oak advance reproduction but optimal light levels remain unknown this species. Care must be taken to achieve release of oak without encouraging the growth of competing vegetation.

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