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Age, growth, longevity, and post-fire/thinning response of chinkapin oak seedlings in a Kansas upland hardwood forest¹

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Abstract. Thinning and prescribed fire are often used to promote oak recruitment into the canopy; however, little is known about the longevity of resprouting oak seedlings and how age affects growth response to these treatments. We investigated this by excavating 180 chinkapin oak seedlings (*Quercus muehlenbergii*) from an eastern Kansas upland hardwood forest study area divided into different combinations of burning and thinning treatments: control, burn only, burn and thin to 7 m²/ha, burn and thin to 14 m²/ha, thin to 7 m²/ha, and thin to 14 m²/ha. In each treatment, approximately three years post-treatment, we aged the seedlings at the root collar (“root age”) and the base of the current aboveground shoot using tissue staining and microscopy techniques. Annual shoot growth rate was calculated for each individual based on the age of the shoot and its total height. We compared these shoot growth rates in height across treatments and investigated this relationship as a function of plant age using the root age. The oldest root age was 48 yr, and 20% of the roots were ≥ 18 yr. Mean root age of advance reproduction was 11.8 years, while mean age of associated aboveground stems was 4.1 yr, with the oldest age structure belonging to treatments without any burn with the control being the oldest. The majority (90%) of the mismatch between root and shoot age was associated with burn treatments. Root age had a strong positive correlation with shoot growth rate in the burn + thinning treatments and also the untreated control, suggesting that age becomes an influence on growth when resprouting under adequate light conditions but also when individuals experience low competition. Further determination of root age structures, such as along gradients of site productivity, would likely aid in further understanding regeneration dynamics and management for sustaining oaks.

Key words: advanced reproduction, chinkapin oak, fire, resprouts, seedlings, tree rings

Overstory oak species are commonly being replaced by nonoak species in a significant part of the world’s more closed-canopy oak forests (Thomas *et al.* 2002; Dey 2014; Imanyfar *et al.* 2019; Spînu *et al.* 2020). The major drivers of this phenomenon are considered to be climate and

pathogen persistence (Thomas *et al.* 2002; Fan *et al.* 2012; Haavik *et al.* 2015; Reed *et al.* 2019), deer browsing (Blossey *et al.* 2019), and altered disturbance regimes, particularly fire exclusion (Abrams and Downs 1990; Guyette *et al.* 2012; Spînu *et al.* 2020). Throughout the eastern United States, for example, closed-canopy oak forests are transitioning to more shade-tolerant species, such as red maple (*Acer rubrum* L.), sugar maple (*Acer saccharum* Marsh.), American beech (*Fagus grandifolia* Ehrh.), and other species (Abrams 1992; Nowacki and Abrams 2008; Alexander *et al.* 2021). Succession of these stands away from oak dominance to mesophytic species is largely expected and predictable (Prasad *et al.* 2007). Oaks are declining in importance due to the success of mesophytic species in outcompeting oaks in regeneration layers of these low-light, closed-canopy conditions (Oswalt *et al.* 2008; Fei *et al.* 2011; Dey 2014). The decline in the growth of oak regeneration in these conditions is largely due to oak’s relative intolerance of shade (Dillaway *et al.* 2007). Further elucidating the growth of the oak regeneration layer is paramount for predicting the possibility of further declines of oak

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importance in forests composed of high oak composition.

Oak seedling resprouts, also referred to as advance reproduction in forestry, consists of the population of understory trees (e.g., ≤ 1 m tall) that have older root stocks, many of which have experienced recurrent shoot dieback. These resprouts can survive decades under a closed canopy when sufficient light periodically exists for growth (Curtis 1959; Tryon and Powell 1984; Crow 1992; Heggenstaller *et al.* 2012), increasing root carbon reserves between disturbances that cause topkill, thereby possibly increasing subsequent sprouting capacity and shoot growth potential (Lorimer 1993; Brose and Van Lear 2004; Dillaway *et al.* 2007; Brose *et al.* 2008). Compared to many shade-tolerant mesophytic species, this adaptation increases oak competitiveness in regeneration and their ability to recruit into the overstory in environments characterized by frequent and low-to moderate-intensity disturbances, such as surface fires (Lorimer 1993; Dey 2014). Many shade-tolerant mesophytic species generally are not as well adapted as oaks to persist and prosper in moderate- to high-frequency, mixed-severity disturbance regimes (Curtis and Rushmore 1958; Burns and Honkala 1990; Alexander *et al.* 2021). Particularly with fire adaptations, mesophytic species commonly have smaller amounts of bark tissue around their base compared to more fire-adapted oaks (Hammond *et al.* 2015).

The amount and distribution of larger-sized oak seedlings (*i.e.*, root collar size), defined in practice by basal diameter, in a given stand are important stand characteristics for gauging future oak regeneration success and sustainability (Loftis 1983; Dey *et al.* 1996; Belli *et al.* 1999; Arthur *et al.* 2012; Dey 2014). Larger-sized seedlings have higher relative height and radial growth rates after disturbance (Dey *et al.* 1996; Belli *et al.* 1999; Dey 2014), likely because of greater nonstructural carbohydrates that enhance resprouting found in roots (Olano *et al.* 2006), though this still needs to be confirmed in some situations (Dillaway *et al.* 2007). In conjunction with root collar size, age is also important for calculating height and radial growth rates and understanding the overall potential for competitive oak regeneration.

Aging of woody species using tree-ring methods has focused primarily on aboveground stems (Cook and Kairiukstis 2013; LeBlanc and Stahle

2015). However, for species that experience repeated shoot dieback and subsequent resprouting (e.g., advance reproduction), stem ages at the root collar may be considerably older than the aboveground stem. Contrary to common dendrochronological studies where aboveground stems can be aged accurately with low-power magnification, accurate aging of the root collar often requires the ability to view and contrast anatomical details achieved by a more involved process, such as tissue microsectioning and staining (Schweingruber *et al.* 2011; Gärtner and Schweingruber 2013). With this approach, absolute dating may be achievable, providing true ages and the ability to determine age effects on aboveground growth, especially in relation to timing of disturbances and management treatments.

The objectives of this study were to use dendrochronology and microsectioning techniques to investigate the age and growth of chinkapin oak (*Quercus muehlenbergii* Engelm.) seedling stems and roots in an eastern Kansas open-canopy hardwood forest with an established burning and thinning experiment (described in more detail in the next section). This study site provided the opportunity to investigate the relationship between age and annual shoot growth of oak seedlings under a suite of light conditions brought on by the thinning and burning experiment. Further, chinkapin oak, being one of the dominant oak species at the site, can be utilized for its relatively high shade intolerance, making it useful for elucidating oak advance reproduction ecology in more closed-canopy forests with a large makeup of shade-intolerant oak species.

Materials and Methods. **SITE DESCRIPTION.** Study sites were located in southeastern Kansas at the Marais des Cygnes Wildlife Area (MDC, 38°15'25.7"N, 94°40'59.9"W). MDC is a 3,100-ha area managed by the Kansas Department of Wildlife, Parks, and Tourism and consists of wetlands, prairies, and a mixture of bottomland and upland hardwood forests. MDC is located within the Wooded Osage Plain physiographic region of Kansas (McNab and Avers 1994; Chapman *et al.* 2001), which is part of the broader Forest-Prairie Transition ecoregion (Bailey 1995, 1997). The climate is temperate, humid, and continental, characterized by large daily and annual variations in temperature. Between 1981 and 2010, the average annual air temperature was

12.9 °C, with an average daily temperature of -1.1 °C in January and 25.7 °C in July (NOAA website, accessed June 15, 2019). During that same period, average annual precipitation was 1,017 mm, with heaviest precipitation occurring in late spring and early summer.

Study sites were located in upland forests with dominant overstory tree species, including chinkapin oak (*Quercus muehlenbergii*), black oak (*Quercus velutina*), bur oak (*Quercus macrocarpa*), ash (*Fraxinus* spp.), and shagbark hickory (*Carya ovata*), and understories dominated by sugar maple, eastern redbud (*Cercis canadensis*), and red elm (*Ulmus rubra*). Dominant oaks have canopy architectures suggesting more open forest conditions in the past, such as wide spreading crowns and low primary branches. The forest is currently closed canopy with an average stem density and stocking of approximately 20 m²/ha and 80%, respectively (Gingrich 1967). The study site is approximately 15 ha, including ridge, shoulder, and backslope topographic positions with elevations ranging from 260 to 310 m and 0–45% slopes. Soils are predominantly moderately deep and well-drained Mollisols of the Claeson series with numerous limestone rock outcrops (Web Soil Survey Staff 2017). Old fences in the area suggest that prior use may have included livestock grazing. The site was burned in a wildfire in the 1950s, and a prescribed fire with unknown coverage occurred in the vicinity in 2005.

STUDY DESIGN AND FIELD SAMPLING. This study was conducted within an experiment that assesses separate and combined effects of thinning and prescribed fire treatments to restore oak woodlands and promote oak and hickory regeneration (Short *et al.* 2019). Treatments include prescribed fire only (B), thin to approximately 14-m²/ha basal area (T14), thin to 7-m²/ha basal area (T7), prescribed fire with each of the thinning treatments (BT14 and BT7, respectively), and an untreated control (C). Treatments were randomly assigned to six 2.5-ha units. Within each treatment, six 0.08-ha vegetation plots were established ($n = 36$) to measure treatment effects on understory plant composition and overall forest structure (Fig. 1). Thinning treatments included overstory and midstory tree girdling with herbicide application in the winter of 2016 and prescribed fire treatments conducted in March 2016. The fire treatment consisted primarily of low-intensity backing fire with average flame

heights of 0.3–0.9 m and rates of spread ranging from 39 to 138 cm/min (Short *et al.* 2019).

In May and September 2018, five chinkapin oak seedlings (trees < 1.5 m tall) were sampled at each vegetation plot ($n = 180$). Resprouts were collected just outside of the plot boundary to avoid disrupting the original study. Seedlings were located by running a transect from the plot center along the azimuths of 70°, 140°, 210°, 280°, and 350° beyond the plot boundary. Once beyond the plot boundary, the first seedling encountered within a 15-m distance from both sides of the transect was excavated with at least the first ~ 10 cm below and including the root collar and all shoots intact (Fig. 1). We used a minimum 10-cm height as a cutoff for including seedlings in the study. If no seedling was found on a given azimuth, then double the number were located on the next azimuth. Seedlings were excavated by shovel and then transported to the Missouri Tree-Ring Laboratory at the University of Missouri for processing.

LABORATORY METHODS. Collected chinkapin oak seedlings were immediately processed in the lab following field excavation. First, all were cleaned of remaining soil with water. Diameter (mm) was measured at the base of the shoot (tallest shoot if multiple) and the top of the root collar, just below the root crown where all shoots originate. The length (assumed *in situ* height in the field) of the tallest shoot was measured to the nearest 0.1 cm. After measurements were made, approximately 5 cm of root below the root collar and 5 cm of stem above the root collar were harvested. The two pieces were placed in a sealable bag with distilled water and stored at 3 °C until subsequent stem microsectioning was undertaken.

We used standard microsectioning and staining techniques (Dietz and Ullman 1998; Dee and Stambaugh 2019) to count and measure annual growth rings. The 5-cm root blocks were first boiled for 30 sec in water to dissolve starches contained in ray cells and improve subsequent staining quality. Next, on the surface closest to the top of the root collar (and, simultaneously for shoots, the surface closest to the root collar), microsectioning was done using a sledge microtome. Microsections of approximately 15 μ in thickness with little to no disruption in cellular anatomy of the cross section were used for staining. To further enhance staining, microsections were bleached for 5–7 min to dissolve

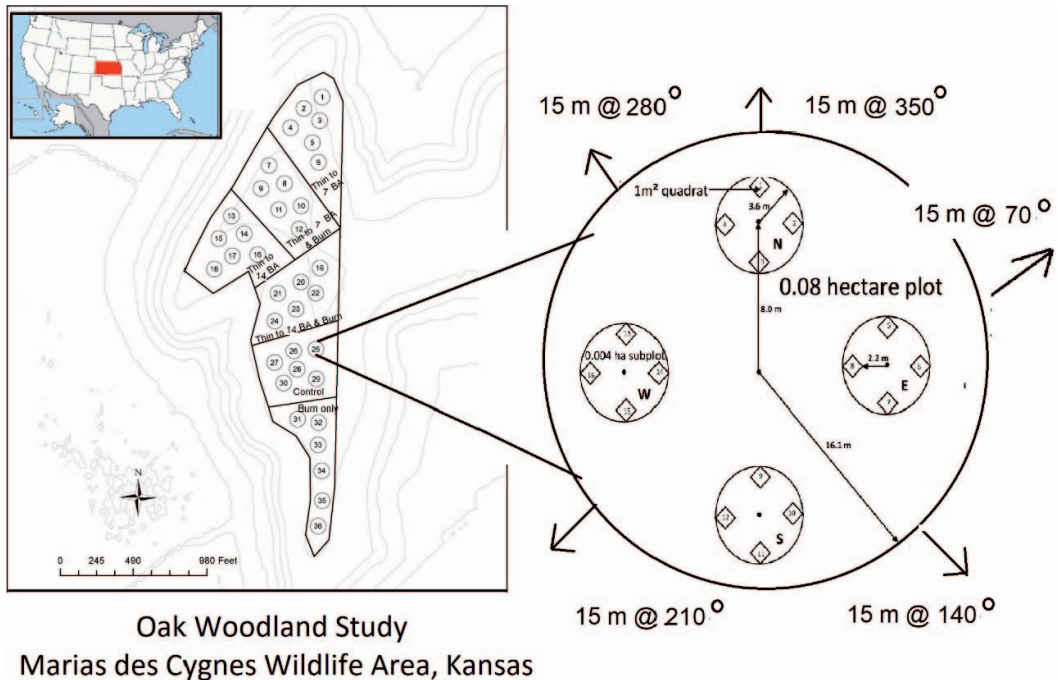


FIG. 1. Orientation of the six treatments that were a combination of burning and thinning to different basal areas (BA) at Marais des Cygnes Wildlife Area in eastern Kansas. Each treatment had six plots nested within each for a total of 36 plots at the site. Plot (0.08 ha) layout also shown; five seedlings were collected up to 15 m beyond the original plot boundary along transects at 70°, 140°, 210°, 280°, and 350° azimuths from plot center.

phenols released during wood decay (Gärtner and Schweingruber 2013) and then washed with water. Two drops of a 1:1 mix of Astra blue and safranin were used for staining (Schweingruber *et al.* 2011), which enhances ring borders by coloring parenchyma cells blue and highly lignified cells red. Microsections sat in staining solution for approximately 2 min and then were washed with 95% ethanol to rid them of excess stain and partially dehydrate them for better optical clarity once mounted on glass slides with Canada Balsam. In most cases, only the root microsection was stained for each seedling; shoot microsections usually had earlywood vessels large enough to easily distinguish ring boundaries and anatomy without staining.

Digital images were taken of whole microsections of both the root and the shoot for each chinkapin oak seedling using a compound microscope set to 40 × magnification and a mounted camera. PTGui 10 software was used to stitch images and make a 1:1 scale image. Using ImageJ software (Abramoff *et al.* 2004), images were scaled by finding the pixel distance of a 1-mm scale of imaged ruler under the same 40 ×

magnification. Before counting and measuring growth rings in images, a protocol was developed to consistently classify ring boundaries (mainly in the stained root microsections) based on cellular anatomy (Fig. 2, Appendix Fig. 1, 2). Ring boundaries were characterized primarily by narrow lignified fiber cells that stained a deep red and that were followed by large earlywood vessels of the next growing season. Further identifiers included high density of larger vessels that shifted in radial arrangement from the previous ring. Ring boundaries were traced using the freehand line tool in ImageJ. The number of ring boundaries immediately after the pith through to the outermost ring was summed to obtain age. Annual ring width was measured along four radii and then averaged.

AGE ANALYSIS, SHOOT HEIGHT GROWTH RATE, AND RADIAL GROWTH OF THE ROOT AND SHOOT. Establishment age (also referred to as root age and “establishment year” in the context of a calendar year associated with germination) was the number of rings from the center primary xylem in roots outward to the edge. The age of the shoot was determined by counting rings from the pith

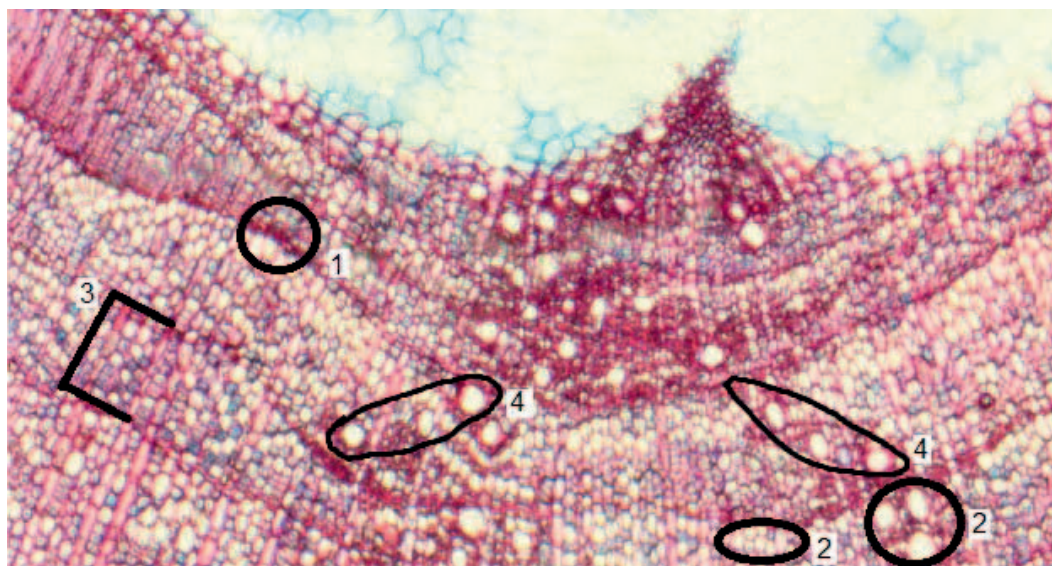


FIG. 2. List of anatomical patterns (in order of weight to make a decision) used to distinguish ring boundaries in images of chinkapin oak seedlings stained root sections; vertical scale of whole image is equal to 1 mm. (1) Thin red line: flattened row of fiber cells one to three cell layers thick with deeply lignified secondary walls (deep red color); tends to be directly before the distinctive large earlywood vessels of the next ring. The line may be broken in spots, but the key is that it follows the general curvature of the section. (2) Large earlywood vessels along with high overall vessel density: occurs after the thin red line. If it is visually present, there is a large shift in the density of vessels (cells with thin walls, large lumens, and no stain). Some of these vessels are large, which is the common characteristic of earlywood vessels. (3) Red, white, and blue: the annual growth ring that follows the red (the thin red line of the latewood of the previous year ring), white (high density of earlywood vessels), and blue (gelatinous fibers of the latewood stage). (4) Vessel grouping directional shift: a good indication of the ring border that occurs when the angle of radial direction of growth shifts to a new direction immediately after the new ring's border.

outward. With these data, we calculated the number of individuals established by calendar year and the difference between establishment age and shoot age of the tallest stem. For trees within the burn treatments, we calculated the percentage of individuals that resprouted in 2016, assuming fire treatments were the main cause of shoot dieback (shoot age therefore would be 3 yr), and created a stacked histogram in SPSS 16 (SPSS Inc. 2007) to visualize these patterns and trends in germination rates in respective years. Finally, we tested the effect of burning and thinning treatments on mean establishment age and shoot age using two separate one-way analyses of variance to test for significant differences across treatments. Because we conducted work at one site, our study is pseudoreplicated at the level of treatment. However, our azimuth-based sampling method within plots of each treatment enabled collection from diverse shade and moisture microenvironments so

that considerable variation in these factors was enforced.

The effect of establishment age on growth metrics among the burning and thinning treatments was investigated. First, we calculated average annual shoot growth rate by dividing maximum height of the tallest shoot by the age of the shoot. Second, we calculated average diameter (radial) growth rates at the root collar and shoot base by dividing these radial diameters by their respective ages. Overall, this analysis involved examining how average individual height growth rate, root collar radial growth rate, and shoot base radial growth rate correlated with root age (establishment age) among the different burning and thinning treatments. Only individuals that were younger than 21 yr old were used in all of these analyses in an effort to balance the sample sizes of ages used among the treatments. Correlations were performed using R 3.4.3 (R Team 2017).

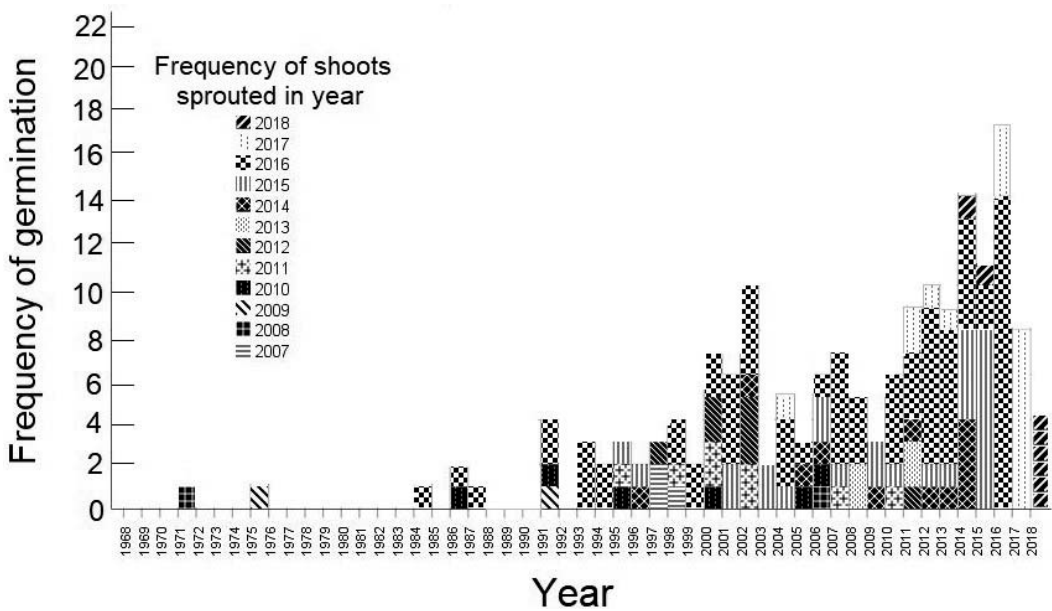


FIG. 3. Stacked histogram highlighting the overall difference between seedling root age with age of the tallest shoot. Each year ("establishment year") has a frequency bar representing the number of seedlings germinated in that year based on root age. Stacked bars represent the frequency of ages of shoots ("sprout year") from all the individuals included in that respective establishment year. Not shown are the associations with treatments, where 90% of seedlings resprouted in response to burning in the spring of 2016.

Results. AGE DISTRIBUTION OF CHINKAPIN OAK ROOTS AND SHOOTS AMONG TREATMENTS. A final sample size of 171 chinkapin oak seedlings was obtained after processing, sectioning, and staining the original samples. The loss of nine individuals was due to decay in the roots that precluded microsectioning. For the nine individuals that were rejected, the average root collar diameter was about 2 mm, suggesting that these seedlings were between 2 and 3 yr old based on the relationship between age and width of the root collar below. For the 171 chinkapin oak seedlings, mean age of the root was 11.8 yr (SD = 8.8), and mean age of the tallest shoot was 4.1 yr (SD = 2.3). Stacked histograms of root age and shoot age show the difference between establishment age and shoot age (Fig. 3). Regardless of root age, 90% of the seedlings within all burn treatments resprouted the year of the fire, and therefore 46% of all 171 shoots were 3 yr old at the time of the study (Fig. 3).

The oldest seedling was 48 yr in root age. Of all 171 seedlings, 20% of the rootstocks were older than 18 yr (established before 2001), 40% were older than 12 yr (established before 2007), 60% were older than 6 yr (established before 2013), and

80% were older than 3 yr (established before 2016). Nine percent of the remaining 20% of sampled trees established in 2016; all but one were in a burn treatment. Three of the seedlings had a maximum shoot age of 12 yr. Overall, 41% of shoots were greater than 3 yr old (sprouted before 2016), 46% were 3 yr old (23% of which were in a treatment that was not burned in 2016), and the remaining 13% sprouted in either 2017 or 2018. Root age and shoot age were significantly different among treatments ($P < 0.001$, $F = 5.26$ and 8.32 ; Fig. 4a, b, respectively). All treatments with burning had significantly younger shoots with a mean of 3.2 yr (SD = 2.3), while all without burning had a mean age of 5.1 yr (SD = 2.4) (Tukey *post hoc*, $P < 0.001$; Fig. 4b).

AGE RELATION WITH CHINKAPIN OAK SEEDLING SHOOT HEIGHT AND RADIAL GROWTH RATES AMONG TREATMENTS. For all 171 seedlings, root age was positively correlated with shoot age ($r = 0.51$, $P < 0.001$) and height of the tallest shoot ($r = 0.57$, $P < 0.001$). The number of shoots was weakly positively related to root age ($r = 0.40$, $P < 0.001$). Root age was strongly correlated with the diameter of the root collar ($r = 0.80$, $P < 0.001$) and the

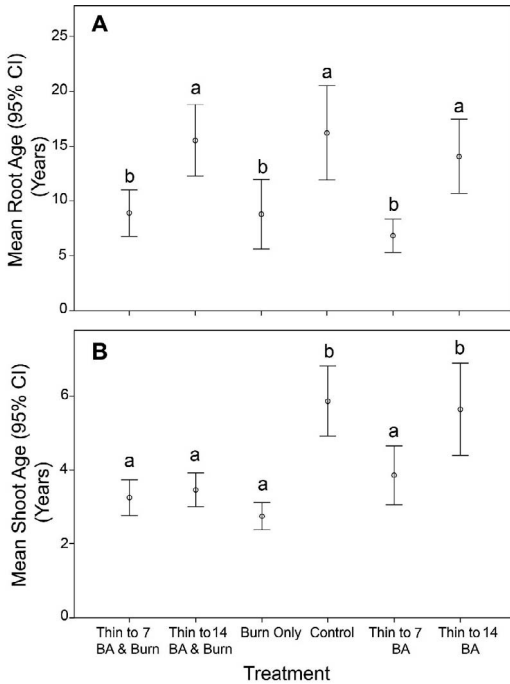


FIG. 4. Mean root age (A) and mean shoot age (B) by treatment for chinkapin oak seedlings. Lines bracket the 95% confidence intervals. Letters that are different from each other represent significant pairwise differences based on Tukey's *post hoc* comparisons for one-way analysis of variance.

number of former shoots based on scars on the root collar ($r = 0.73$, $P < 0.001$).

Dependent on treatment, we found a significant positive relationship between root age, height, and radial growth rates (see Table 1 for mean values between treatments). The positive significant effect of root age on shoot height growth rates was pronounced in the burn + thinning treatments: $r = 0.48$, $P = 0.015$, for BT7 and $r = 0.70$, $P < 0.001$, for BT14 (Fig. 5a). However, for the untreated control treatment, there was also a positive significant relationship: $r = 0.47$, $P = 0.029$. BU, T7, and T14 all do not have significant relationships between root age and shoot height growth rates. The relationship between root caliper width and shoot height growth rate show the same patterns of significance— $r = 0.54$, $P = 0.005$, for BT7; $r = 0.84$, $P = 0.001$, for BT14; and $r = 0.51$, $P = 0.014$, for the control—and once again no significant relationships were seen with the burn-only and thin-only treatments (Fig. 5b).

There was a significant positive relationship between root age and shoot radial growth rate in

Table 1. Mean height and radial growth rate (cm and mm/yr, respectively) of shoots between treatments. BA = basal area.

Treatment	Mean height shoot growth rate (cm/yr)	Mean shoot radial growth rate (mm/yr)
Thin to 7 BA and burn	10.34	1.65
Thin to 14 BA and burn	10.95	1.32
Burn only	5.57	0.97
Control	6.22	0.98
Thin to 7 BA	9.33	1.39
Thin to 14 BA	6.82	1.20

the burn + thinning treatments (Fig. 5c)— $r = 0.51$, $P = 0.010$, for BT7 and $r = 0.54$, $P = 0.012$, for BT14—but this significant relationship is not present in the control treatment. Likewise, the burn-only and thin-only treatments yielded no significant relationships. Finally, there was still a significant positive effect of root caliper width on shoot radial growth rate in the burn + thinning treatments— $r = 0.64$, $P = 0.001$, for BT7 and $r = 0.66$, $P = 0.001$, for BT14—but also a weak significant positive relationship in the burn-only treatment— $r = 0.43$, $P = 0.034$ (Fig. 5d).

Discussion. AGE DISTRIBUTION OF CHINKAPIN OAK SEEDLINGS, REGENERATION POTENTIAL, AND MANAGEMENT IMPLICATIONS. Using microsectioning techniques, we were able to quantify the age of oak seedling roots and shoots, a rare trait utilized in relevant oak ecology studies. With ages of roots and shoots, we were able to gauge the actual age distribution and longevity of seedlings at our open-canopy, xeric Kansas study site. We found that chinkapin oak seedlings can persist in the understory for several decades based on about 20% of our samples being at least 20 yr old. In total, 90% of seedlings resprouted after the 2016 fire regardless of root age. Although site comparison was not a focus of our case study, our findings provide support that the establishment of larger/older oak seedlings has the potential to accumulate on less productive, open-canopy, xeric sites represented by this Kansas study (*sensu* Johnson *et al.* 2019). Canopy recruitment potential of shade-intolerant oaks on these sites could be high because these older/larger seedlings are relatively competitive compared to younger and smaller individuals (Brose and Van Lear 2004; Arthur *et al.* 2012; Dey 2014). The significant positive correlations we found between root age, height, number of shoots, and root collar diameter are all

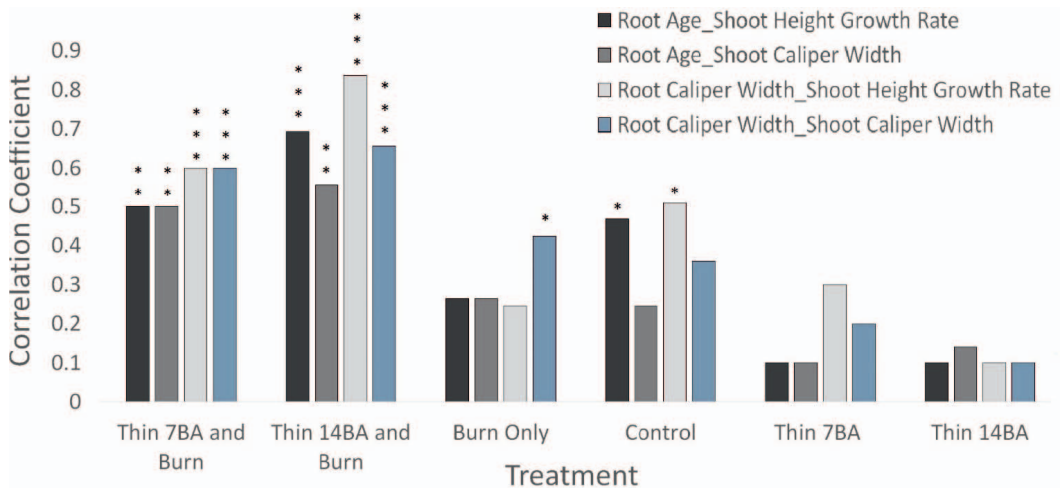


FIG. 5. Correlation coefficients between different shoot growth metrics for either root age or root collar diameter among the different treatments. Only chinkapin oak seedlings that were less than 21 yr old at the time of excavation were used in this analysis (84% of the total sample) for the purpose of keeping approximately equal representation of ages (1–20) in each treatment. * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.

attributes of seemingly more competitive seedlings able to eventually recruit into the canopy (Brose and Van Lear 2004; Arthur *et al.* 2012; Dey 2014). Conversely, on more mesic sites, typical of more closed-canopy oak forests, oak regeneration failure is common (Ward and Stephens 1994; Aldrich *et al.* 2005; Gotmark *et al.* 2005; Pulido and Diaz 2005; Morrissey *et al.* 2008) because increases in mesophytic species dominance decreases a substantial amount of light to the understory, resulting in reduced abundance and competitiveness of shade-intolerant oak seedlings such as chinkapin oak (Aldrich *et al.* 2005; McEwan and Muller 2006; Oswalt *et al.* 2008; Fei *et al.* 2011).

Fire regimes have a substantial effect on canopy structure that could affect the composition and longevity of shade-intolerant oak seedlings near the forest floor. Although we do not have documented fire history for our site beyond 50 yr, recurring frequent fires were a historical disturbance in this region, causing many mesic sites to be more open structured than they are today (Stambaugh *et al.* 2014). Considering the past century of fire exclusion in this region and many other oak forests across the world (Stambaugh and Guyette 2006; Guyette *et al.* 2012; Stambaugh *et al.* 2014; Spínu *et al.* 2020), a return to a frequent fire regime resulting in more open midstory and overstory conditions conducive to accumulation of older, larger, and more competitive oak seedlings may be key to providing a

reversal of the oak regeneration problem in many mesic closed-canopy sites (Ward and Stephens 1994; Aldrich *et al.* 2005; Gotmark *et al.* 2005; Pulido and Diaz 2005; Morrissey *et al.* 2008; Dey 2014).

AGE-RELATED GROWTH RESPONSE TO DISTURBANCE. We found evidence for a strong positive relationship between root age and annual shoot height growth rates after implementation of the burning and thinning treatments in 2016. The positive correlation between root age and height growth rate was strongest in the thinning and burning treatments, with BT14 having the strongest positive relationship. Relatively high correlations were also found between root collar diameter and height growth rate, which were highest in the thinning and burning treatments. Increased height growth rate associated with increasing root collar diameter underscores the importance of accumulating larger oak reproduction to increase their competitiveness. Larger root collar caliper is significantly correlated with root size, hence the possibility of high root carbohydrate reserves (Dey and Parker 1997; Olano *et al.* 2006; Dillaway *et al.* 2007; Brose *et al.* 2008). Most positive associations between age and growth rate existed for all treatments where the canopy was thinned, highlighting the importance of canopy-opening events that substantially increase light in the understory (Abrams *et al.* 1995; Soucy *et al.* 2005; Schweitzer

and Dey 2011; Hutchinson *et al.* 2012; Brose *et al.* 2013; Dey 2014). However, it appears that fire accompanying thinning was important for promoting oak release under an opened canopy because thin-only treatments showed no significant relationship between age and height growth rate after treatment.

Prescribed fire reduced the density of midstory stems and reduced, at least in the short term, the understory canopy of woody trees, shrubs, and vines at our site (Short *et al.* 2019). In many closed-canopy oak forests where competition from fire-intolerant mesophytic species limits oak regeneration (Abrams 1992; Nowacki and Abrams 2008), fires contribute to oak growth by reducing competing vegetation. Many common oak seedling competitors, such as sugar maple and other mesophytic species, have lower survival and resprout vigor following fire topkill, especially in a frequent fire regime (Brose and Van Lear 2004; Brose *et al.* 2013; Johnson *et al.* 2019), though results can be mixed with red maple, for example, also remaining competitive with oak in frequent fire (Green *et al.* 2010). Brose and Van Lear (2004) and Brose *et al.* (2013) reported that oak stem density several years postfire was usually greater than the density of each competing mesophytic species, especially where greater fire intensity occurred in more open canopy conditions. Without fire, thinning alone helps increase the density of many competitors to oak (Schweitzer and Dey 2011; Hutchinson *et al.* 2012; Short *et al.* 2019). This could be the reason why we unexpectedly saw a weaker but sustained significant relationship between chinkapin seedling age and growth in the control plots and no relation in the burn-only plots, where in both conditions, only low densities of competitors exist in the low understory light conditions (Short *et al.* 2019). It is possible that under low competition, a chinkapin seedling age-growth relationship is sustained, as we saw, since the areas with the highest density of competitors (thin-only treatments) possessed an age-growth relationship that was very weak or nonexistent.

Besides controlling competitors after canopy-opening events, fire is also important in oak regeneration by stimulating new growth via resprouting under an increased light environment. Since oaks preferentially allocate carbohydrates to the roots and thereby accumulate energy reserves with age (Crunkilton *et al.* 1992; Dey and Parker 1996; Dillaway *et al.* 2007), older oak reproduc-

tion that resprouts after fire could have a relative growth advantage over species that invest growth in shoot biomass, which can be lost due to fire topkill. Our observation of a positive association between seedling age and our various metrics for shoot growth rates, which was highest in thinning and burning treatments, has been confirmed by others who have observed increased competitive ability of oak advance reproduction that have larger base diameter of shoots (Dey *et al.* 1996; Belli *et al.* 1999; Dey 2014). Brose and Van Lear (1998) documented the relatively faster growth rates of new oak shoots in post-fire conditions, which often were higher than established shoots that were not exposed to fire. Overall, it is common to see enhanced growth of oak reproduction following fire and canopy openings, along with other shade-intolerants (Schweitzer and Dey 2011; Hutchinson *et al.* 2012). However, this association is not always clear, as several studies have found no difference in oak regeneration growth or mortality following fire compared to unburned conditions likely due to various factors such as pre-burn size, variation in fire behavior, and fire frequency (Alexander *et al.* 2008; Keyser *et al.* 2017).

Conclusions. We used microsectioning techniques to age oak advance reproduction roots and shoots at a site with different canopy and fire treatment combinations. This approach allowed us to quantify the age distribution of oak advance reproduction, a rare parameter present in similar studies. With ages of roots and shoots, we were able to analyze annual growth rates of our oak seedlings under different management treatments. We found that the open-canopy conditions created by thinning and burning treatments (Short *et al.* 2019) seemed to be advantageous for growth of older oak seedlings provided that there were older and larger chinkapin oak seedlings present in the understory before treatment. Our study was small in scale; thus, we caution the extrapolation of our results to regeneration in other oak forests and species. Many site-related factors affect the availability of older oak advance reproduction, including whether sites are accumulators or recalcitrant (Dey 2014; Johnson *et al.* 2019). Nevertheless, we suggest that our approach to oak advance reproduction aging and growth analysis should be considered for future studies across a wider array of oak forests and species

since a better understanding of age distributions and age-related growth responses will likely benefit oak regeneration success and inform restoration and management of these ecosystems.

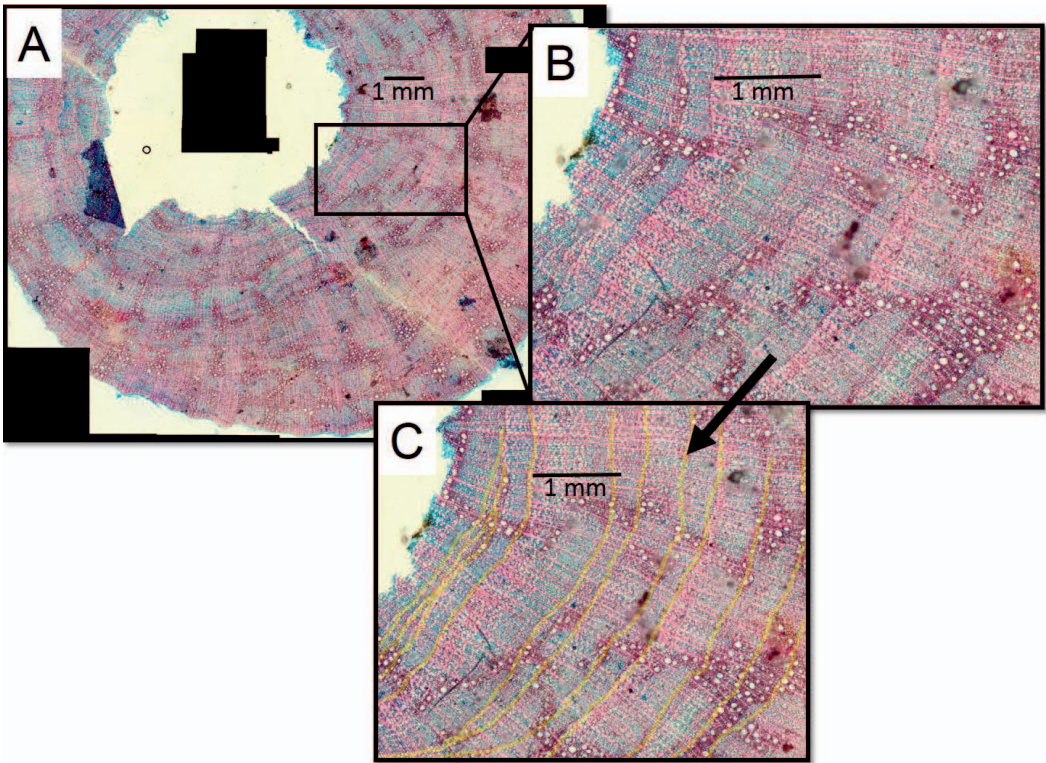
Literature Cited

- ABRAMOFF, M. D., P. J. MAGALHAES, AND S. J. RAM. 2004. Image processing with ImageJ. *Biophotonics International* 11: 36–42.
- ABRAMS, M. D. 1992. Fire and the development of oak forests. *BioScience* 42: 346–353.
- ABRAMS, M. D. AND J. A. DOWNS. 1990. Successional replacement of old-growth white oak by mixed mesophytic hardwoods in southwestern Pennsylvania. *Canadian Journal of Forest Research* 20: 1864–1870.
- ABRAMS, M. D., D. A. ORWIG, AND T. E. DEMEO. 1995. Dendroecological analysis of successional dynamics for a presettlement-origin white-pine-mixed-oak forest in the southern Appalachians, USA. *Journal of Ecology* 83: 123–133.
- ALDRICH, P. R., G. R. PARKER, J. ROMERO-SEVERSON, AND C. H. MICHLER. 2005. Confirmation of oak recruitment failure in Indiana old-growth forest: 75 years of data. *Forest Science* 51: 406–416.
- ALEXANDER, H. D., M. A. ARTHUR, D. L. LOFTIS, AND S. R. GREEN. 2008. Survival and growth of upland oak and co-occurring competitor seedlings following single and repeated prescribed fires. *Forest Ecology and Management* 256: 1021–1030.
- ALEXANDER, H. D., C. SIEGERT, J. S. BREWER, J. KREYE, M. A. LASHLEY, J. K. MCDANIEL, A. K. PAULSON, H. J. RENNIGER, AND J. M. VARNER. 2021. Mesophication of oak landscapes: Evidence, knowledge gaps, and future research. *BioScience* 71: 531–542.
- ARTHUR, M. A., H. D. ALEXANDER, D. C. DEY, C. J. SCHWEITZER, AND D. L. LOFTIS. 2012. Refining the oak-fire hypothesis for management of oak-dominated forests of the eastern United States. *Journal of Forestry* 110: 257–266.
- BAILEY, R. G. 1995. Descriptions of the Ecoregions of the United States. USDA Miscellaneous Publication 1391. United States Department of Agriculture, Washington, DC. 77 pp.
- BAILEY, R. G. 1997. Ecoregions of North America, 1:15,000,000 Scale Map (Rev.). United States Department of Agriculture, Forest Service, Washington, DC.
- BELLI, K. L., C. P. HART, J. D. HODGES, AND J. A. STANTURF. 1999. Assessment of the regeneration potential of red oaks and ash on minor bottoms in Mississippi. *Southern Journal of Applied Forestry* 23: 133–138.
- BLOSSEY, B., P. CURTIS, J. BOULANGER, AND A. DÁVALOS. 2019. Red oak seedlings as indicators of deer browse pressure: Gauging the outcome of different white-tailed deer management approaches. *Ecology and Evolution* 9: 13085–13103.
- BROSE, P. H., D. C. DEY, R. J. PHILLIPS, AND T. A. WALDROP. 2013. A meta-analysis of the fire-oak hypothesis: Does prescribed burning promote oak reproduction in eastern North America? *Forest Science* 59: 322–334.
- BROSE, P. H., K. W. GOTTSCHALK, S. B. HORSLEY, P. D. KNOPP, J. KOCHENDERFER, B. J. MCGUINNESS, AND G. W. MILLER. 2008. Prescribing regeneration treatments for mixed-oak forests in the Mid-Atlantic region. General Technical Report NRS-33. United States Department of Agriculture, Forest Service, Northern Research Station, Newtown Road, PA. 100 pp.
- BROSE, P. H. AND D. H. VAN LEAR. 1998. Responses of hardwood advance regeneration to seasonal prescribed fires in oak-dominated shelterwood stands. *Canadian Journal of Forest Research* 28: 331–339.
- BROSE, P. H. AND D. H. VAN LEAR. 2004. Survival of hardwood regeneration during prescribed fires: The importance of root development and root collar location, pp. 123–127. *In* M. A. Spetich, ed. *Proceedings, Upland Oak Ecology Symposium: History, Current Conditions, and Sustainability*; October 7–10, 2002, Fayetteville, AR. General Technical Report SRS-73. United States Department of Agriculture, Forest Service, Asheville, NC. 311 pp.
- BURNS, R. M. AND B. H. HONKALA. 1990. Silvics of North America: Volume 2. Hardwoods. Agriculture Handbook 654. United States Department of Agriculture, Forest Service, Washington, DC. 119 p.
- CHAPMAN, S. S., J. M. OMERNIK, J. A. FREEOUF, D. G. HUGGINS, J. R. MCCAULEY, C. C. FREEMAN, G. STEINAUER, R. T. ANGELO, AND R. L. SCHLEPP. 2001. Ecoregions of Nebraska and Kansas (color poster with map, descriptive text, summary tables, and photographs). U.S. Geological Survey, Reston, VA.
- COOK, E. R. AND L. A. KAIRIUKSTIS. 2013. *Methods of Dendrochronology: Applications in the Environmental Sciences*. Springer Science & Business Media, New York, NY. 406 pp.
- CROW, T. R. 1992. Population dynamics and growth patterns for a cohort of northern red oak (*Quercus rubra*) seedlings. *Oecologia* 91: 192–200.
- CRUNKILTON, D. D., S. G. PALLARDY, AND H. E. GARRETT. 1992. Water relations and gas exchange of northern red oak seedlings planted in a central Missouri clearcut and shelterwood. *Forest Ecology and Management* 53: 117–129.
- CURTIS, J. T. 1959. *The Vegetation of Wisconsin*. University of Wisconsin Press, Madison, WI. 704 pp.
- CURTIS, R. O. AND F. M. RUSHMORE. 1958. Some effects of stand density and deer browsing on reproduction in an Adirondack hardwood stand. *Journal of Forestry* 56: 116–121.
- DEE, J. R. AND M. C. STAMBAUGH. 2019. A new approach towards climate monitoring in Rocky Mountain alpine plant communities: A case study using herb-chronology and *Penstemon whippleanus*. *Arctic, Antarctic, and Alpine Research* 51: 84–95.
- DEY, D. C. 2014. Sustaining oak forests in eastern North America: Regeneration and recruitment, the pillars of sustainability. *Forest Science* 60: 926–942.
- DEY, D. C. AND W. C. PARKER. 1996. Regeneration of red oak (*Quercus rubra* L.) using shelterwood systems: Ecophysiology, silviculture and management recommendations. Ontario Forest Research Institute Forest Research Information Paper 126. Ontario Ministry of Natural Resources, Ontario Forest Research Institute, Sault Ste. Marie, ON, Canada. 59 pp.
- DEY, D. C. AND W. C. PARKER. 1997. Morphological indicators of stock quality and field performance of red oak (*Quercus rubra* L.) seedling underplanted in a

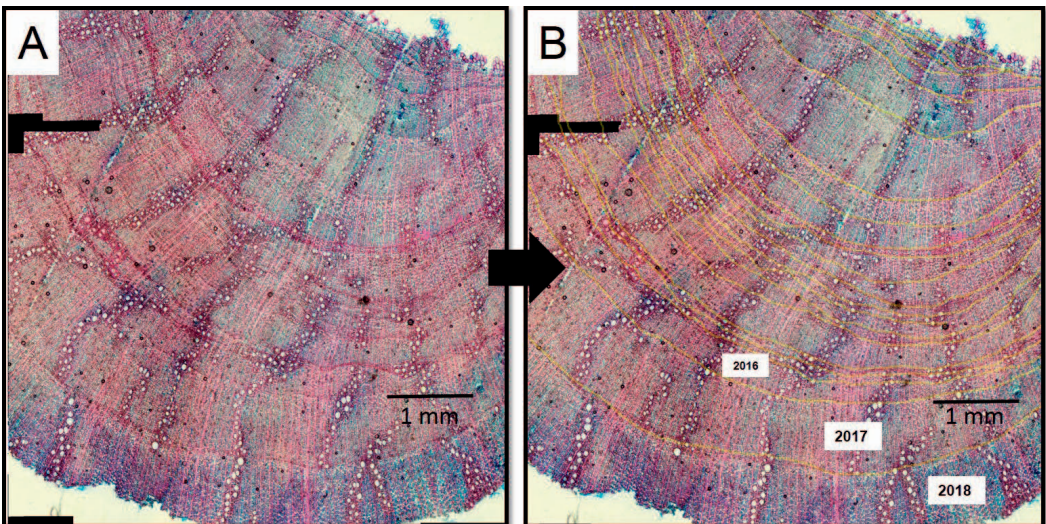
- central Ontario shelterwood. *New Forests* 14: 145–156.
- DEY, D. C., M. TER-MIKAELIAN, P. S. JOHNSON, AND S. R. SHIFLEY. 1996. Users guide to ACORn: A comprehensive Ozark regeneration simulator. General Technical Report NC-18. United States Department of Agriculture, North Central Forest Experimental Station, St. Paul, MN. 35 pp.
- DIETZ, H. AND I. ULLMAN. 1998. Ecological application of “herbchronology”: Comparative stand age structure analyses of the invasive plant *Bunias orientalis* L. *Annals of Botany* 82: 471–480.
- DILLAWAY, D. N., J. W. STRINGER, AND L. K. RIESKE. 2007. Light availability influences root carbohydrates, and potentially vigor, in white oak advance regeneration. *Forest Ecology and Management* 250: 227–233.
- FAN, Z., X. FAN, M. K. CROSBY, W. K. MOSER, H. HE, M. A. SPETICH, AND S. R. SHIFLEY. 2012. Spatio-temporal trends of oak decline and mortality under periodic regional drought in the Ozark Highlands of Arkansas and Missouri. *Forests* 3: 614–631.
- FEL, S., N. KONG, K. C. STEINER, W. K. MOSER, AND E. B. STEINER. 2011. Change in oak abundance in the eastern United States from 1980 to 2008. *Forest Ecology and Management* 262: 1370–1377.
- GÄRTNER, H. AND F. H. SCHWEINGRUBER. 2013. *Microscopic Preparation Techniques for Plant Stem Analysis*. Verlag Dr. Kessel, Remagen, Germany.
- GINGRICH, S. F. 1967. Measuring and evaluating stocking and stand density in upland hardwood forests in the Central States. *Forest Science* 13: 38–53.
- GOTMARK, F., A. BERGLUND, AND K. WILKLANDER. 2005. Browsing damage on broadleaved trees in semi-natural temperate forest in Sweden, with a focus on oak regeneration. *Scandinavian Journal of Forest Restoration* 20: 223–234.
- GREEN, S. R., M. A. ARTHUR, AND B. A. BLANKENSHIP. 2010. Oak and red maple seedling survival and growth following periodic prescribed fire on xeric ridgetops on the Cumberland Plateau. *Forest Ecology and Management* 259: 2256–2266.
- GUYETTE, R. P., M. C. STAMBAUGH, D. C. DEY, AND R.-M. MUZIKA. 2012. Predicting fire frequency with chemistry and climate. *Ecosystems* 15: 322–335.
- HAAVIK, L. J., S. A. BILLINGS, J. M. GULDIN, AND F. M. STEPHEN. 2015. Emergent insects, pathogens and drought shape changing patterns in oak decline in North America and Europe. *Forest Ecology and Management* 354: 190–205.
- HAMMOND, D. H., J. M. VARNER, J. S. KUSH, AND Z. FAN. 2015. Contrasting sapling bark allocation of five southeastern USA hardwood tree species in a fire prone ecosystem. *Ecosphere* 6: 1–13.
- HEGGENSTALLER, D. J., E. K. ZENNER, P. H. BROSE, AND J. E. PECK. 2012. How much older are Appalachian oaks below-ground than above-ground? *Northern Journal of Applied Forestry* 29: 155–157.
- HUTCHINSON, T. F., R. P. LONG, J. REBBECK, E. K. SUTHERLAND, AND D. A. YAUSSY. 2012. Repeated prescribed fires alter gap-phase regeneration in mixed-oak forests. *Canadian Journal of Forest Research* 42: 303–314.
- IMANYEAR, S., M. HASANLOU, AND V. MIRZAEI ZADEH. 2019. Mapping oak decline through long-term analysis of time series of satellite images in the forests of Malekshahi, Iran. *International Journal of Remote Sensing* 40: 8705–8726.
- JOHNSON, P. S., S. R. SHIFLEY, R. ROGERS, D. C. DEY, AND J. M. KABRICK. 2019. *The Ecology and Silviculture of Oaks*, 3rd ed. CABI, Boston, MA. 648 pp.
- KEYSER, T. L., M. ARTHUR, AND D. L. LOFTIS. 2017. Repeated burning alters the structure and composition of hardwood regeneration in oak-dominated forests of eastern Kentucky, USA. *Forest Ecology and Management* 393: 1–11.
- LEBLANC, D. C. AND D. W. STAHL. 2015. Radial growth responses of four oak species to climate in eastern and central North America. *Canadian Journal of Forest Research* 45: 793–804.
- LOFTIS, D. L. 1983. Regenerating red oak on productive sites in the southern Appalachians: A research approach, pp. 144–150. *In* E. P. Jones, ed. *Proceedings, Second Biennial Southern Silvicultural Research Station Conference*, November 4–5, 1982, Atlanta, GA. General Technical Report SE-24. United States Department of Agriculture, Forest Service, Southeastern Forest Experimental Station, Asheville, NC. 526 pp.
- LORIMER, C. G. 1993. Causes of the oak regeneration problem, pp. 14–39. *In* D. Loftis and C. E. McGee, eds. *Oak Regeneration: Serious Problems, Practical Recommendations*. General Technical Report SE-84. United States Department of Agriculture, Forest Service, Southeastern Forest Experiment Station, Asheville, NC. 319 pp.
- MCEWAN, R. W. AND R. N. MULLER. 2006. Spatial and temporal dynamics in canopy dominance of an old-growth central Appalachian forest. *Canadian Journal of Forest Research* 36: 1536–1550.
- MENAB, W. H. AND P. E. AVERS. 1994. *Ecological subregions of the United States: Section descriptions*. Administrative Publication WO-WSA-5. United States Department of Agriculture, Forest Service, Washington, DC. 267 pp.
- MORRISSEY, R. C., D. F. JACOBS, J. R. SEIFERT, B. C. FISCHER, AND J. A. KERSHAW. 2008. Competitive success of natural oak regeneration in clearcuts during the stem exclusion stage. *Canadian Journal of Forest Research* 38: 1419–1430.
- NOWACKI, G. J. AND M. D. ABRAMS. 2008. The demise of fire and “mesophication” of forests in the eastern United States. *Bioscience* 58: 123–138.
- OLANO, J. M., E. S. MENGES, AND E. MARTINEZ. 2006. Carbohydrate storage in five resprouting Florida scrub plants across a fire chronosequence. *New Phytologist* 170: 99–106.
- OSWALT, C. M., J. W. STRINGER, AND J. A. TURNER. 2008. Shifts in relative stocking of common tree species in Kentucky from 1975 to 2004, pp. 194–203. *In* D. F. Jacobs and C. H. Michler, eds. *Proceedings, 16th Central Hardwood Forest Conference*, April 8–9, 2008, West Lafayette, IN. United States Department of Agriculture, Forest Service, Northern Research Station, Newtown Square, PA. 608 pp.

- PRASAD, A. M., L. R. IVERSON, S. MATTHEWS, AND M. PETERS. 2007. A Climate Change Atlas for 134 Forest Tree Species of the Eastern United States [database]. United States Department of Agriculture, Forest Service, Northern Research Station, Delaware, OH.
- PULIDO, F. J. AND M. DIAZ. 2005. Regeneration of a Mediterranean oak: A whole-cycle approach. *Eco-science* 12: 92–102.
- R TEAM. 2017. RStudio: Integrated Development for R. RStudio, Inc., Boston, MA. <http://www.rstudio.com>, 42.
- REED, S. E., J. T. ENGLISH, AND R. M. MUZIKA. 2019. Phytophthora species detected in two Ozark forests with unusual patterns of white oak mortality. *Plant Disease* 103(1): 102–109.
- SCHWEINGRUBER, F. H., A. BÖRNER, AND E. D. SCHULZE. 2011. Atlas of Stem Anatomy in Herbs, Shrubs and Trees. Springer Science & Business Media, New York, NY. 415 pp.
- SCHWEITZER, C. J. AND D. C. DEY. 2011. Forest structure, composition, and tree diversity response to a gradient of regeneration harvests in the mid-Cumberland Plateau escarpment region, USA. *Forest Ecology and Management* 262: 1729–1741.
- SHORT, M. F., M. C. STAMBAUGH, AND D. C. DEY. 2019. Prescribed fire effects on oak woodland advance regeneration at the prairie–forest border in Kansas, USA. *Canadian Journal of Forest Research* 49: 1570–1579.
- SOUCY, R. D., E. HEITZMAN, AND M. A. SPETICH. 2005. The establishment and development of oak forests in the Ozark Mountains of Arkansas. *Canadian Journal of Forest Research* 35: 1790–1797.
- SPINU, A. P., M. NIKLASSON, AND E. ZIN. 2020. Mesophilization in temperate Europe: A dendrochronological reconstruction of tree succession and fires in a mixed deciduous stand in Białowieża Forest. *Ecology and Evolution* 10: 1029–1041.
- SPSS INC. 2007. SPSS for Windows, Version 16.0. SPSS Inc., Chicago, IL.
- STAMBAUGH, M. C. AND R. P. GUYETTE. 2006. Fire regime of an Ozark wilderness area, Arkansas. *The American Midland Naturalist* 156: 237–252.
- STAMBAUGH, M. C., J. M. MARSCHALL, AND R. P. GUYETTE. 2014. Linking fire history to successional changes of xeric oak woodlands. *Forest Ecology and Management* 320: 83–95.
- THOMAS, F. M., R. BLANK, AND G. HARTMANN. 2002. Abiotic and biotic factors and their interactions as causes of oak decline in Central Europe. *Forest Pathology* 32: 277–307.
- TRYON, E. H. AND D. S. POWELL. 1984. Root ages of advance hardwood reproduction. *Forest Ecology and Management* 8: 293–298.
- WARD, J. S. AND G. R. STEPHENS. 1994. Crown class transition rates of maturing northern red oak (*Quercus rubra* L.). *Forest Science* 40: 221–237.
- WEB SOIL SURVEY STAFF. 2017. Web Soil Survey. United States Department of Agriculture, Natural Resources Conservation Service. <https://websoilsurvey.sc.egov.usda.gov>. Retrieved June 15, 2019.

Appendix



APPENDIX FIG. 1. A. Common example of a stained chinkapin oak seedlings root section, several 40 x images stitched together to make a whole-scale image of the cross-section. B. Closer view of cellular anatomy described in Fig. 2 used to distinguish ring borders. C. Ring borders drawn in using ImageJ, showing 12 distinct rings.



APPENDIX FIG. 2. A. Another typical stained root section image stitched together from a series of 40 x images. B. Ring borders drawn in on the same root section, 22 rings shown overall, but highlighting a common occurrence with chinkapin oak seedlings under any thinning, great amount of release from the year (2016) when at least thinning occurred.