Forest Ecology and Management 379 (2016) 146–152

Contents lists available at ScienceDirect

Forest Ecology and Management

journal homepage: www.elsevier.com/locate/foreco

# Post-fire resprouting of shortleaf pine is facilitated by a morphological trait but fire eliminates shortleaf × loblolly pine hybrid seedlings

J.C. Bradley [a], R.E. Will [a,*], J.F. Stewart [b], C.D. Nelson [c,d], J.M. Guldin [e]

[a] Department of Natural Resources Ecology and Management, 008C Agricultural Hall, Oklahoma State University, Stillwater, OK 74078, USA
[b] Department of Integrative Biology, Oklahoma State University, Stillwater, OK 74078, USA
[c] USDA Forest Service, Southern Research Station, Southern Institute of Forest Genetics, 23332 Success Rd., Saucier, MS 39574, USA
[d] Forest Health Research and Education Center, 208 T.P. Cooper Hall, University of Kentucky, Lexington, KY 40546, USA
[e] USDA Forest Service, Southern Research Station, 607 Reserve St., Hot Springs, AR 71902, USA

ARTICLE INFO

*Article history:*
Received 5 July 2016
Received in revised form 8 August 2016
Accepted 9 August 2016
Available online 16 August 2016

*Keywords:*
Basal crook
Fire
*Pinus echinata*
*Pinus taeda*
Resprouting
Shortleaf × loblolly pine hybrid

ABSTRACT

Concurrent with fire exclusion, shortleaf pine × loblolly pine hybrids have increased throughout the southeastern USA and may threaten the genetic integrity of shortleaf pine. Historically, fire favored shortleaf pine over other southern pine species, especially loblolly pine, with which it shares a broad sympatric natural range. Shortleaf pine seedlings have a morphological adaptation (basal crook) that lowers dormant buds to the soil surface where they are presumably protected from fire to facilitate resprouting after topkill. To evaluate this presumption, we tested (1) the functional role of the basal crook in protecting dormant buds by measuring resprouting after exposing the lower stem of shortleaf pine to fire and protecting from fire the lower stem (and dormant buds) of loblolly pine (which lacks a basal crook) and (2) the occurrence and importance of the basal crook morphological adaptation in F1 shortleaf × loblolly pine seedlings by comparing morphology and post-fire resprouting of the hybrids to both parents. Fire exposure of shortleaf pine seedling dormant buds caused seedling mortality, while protecting loblolly pine dormant buds facilitated resprouting. Hybrid pines have basal crooks intermediate to the strong crook of shortleaf pine and loblolly pine's non-crooked stem. Fire top-killed loblolly pine and shortleaf × loblolly pine seedlings, and they did not resprout, while 57% of shortleaf pine resprouted after topkill during two dormant season and one growing season burns. This highly significant difference shows that the basal crook is important for shortleaf pine resprouting after topkill by fire. Fire is an important tool to reduce shortleaf × loblolly pine hybrids, and to provide a competitive advantage to shortleaf pine relative to loblolly pine, for ecosystem restoration and maintaining the genetic integrity of shortleaf pine.


## 1. Introduction

Shortleaf pine (*Pinus echinata* Mill.) has the widest distribution of any pine in the southeastern USA. While the species is broadly distributed, shortleaf pine abundance has decreased over the last century. McWilliams et al. (1986) estimated that shortleaf pine forest type suffered an approximately 50% reduction from the 1950s to the late 1970s, and Oswalt (2011) quantified a loss of an additional half of remaining shortleaf pine forests between the 1980s and 2010. Factors for this decline include the interruption of historic fire patterns, land use change, and replacement of shortleaf pine with planted stands of more productive timber species, especially loblolly pine (*P. taeda* L.) (Tauer et al., 2012). In addition to these threats, shortleaf pine is at risk to hybridization with and introgression towards loblolly pine. Introgression between the loblolly pine and shortleaf pine has increased from 3.3% to 45.7% since the 1950s in shortleaf pine stands when current seedling populations were compared to adult trees originating from the same areas (Stewart et al., 2012). Hybridization may imperil the genetic integrity of shortleaf pine and reduce the resilience of pine seedlings to fire and drought (Tauer et al., 2012).

Fire is historically associated with shortleaf pine forests. In an early account, Mattoon (1915) documents the link between fire and shortleaf pine, "Its range over the drier uplands is coincident with a region of frequent forest fires, yet it is saved by notably abundant reproduction practically everywhere." With prolonged fire exclusion, shortleaf pine is replaced by later successional angiosperm tree species (Guldin, 1986). The most prominent trait of shortleaf pine to survive burning is the ability of seedlings and saplings to vigorously resprout after topkill from fire (e.g., Mattoon, 1915).

* Corresponding author.
*E-mail address:* rodney.will@okstate.edu (R.E. Will).

http://dx.doi.org/10.1016/j.foreco.2016.08.016

A plant species' ability to resprout following topkill from fire is an important attribute for success and persistence in a landscape that experiences periodic fire (Pausas et al., 2016; Pausas and Keeley, 2014). There are a number of traits that facilitate resprouting after fire, including maintaining dormant buds at or below the soil surface where the low thermal conductivity of the soil protects and insulates these buds (Clarke et al., 2013). Other traits that afford protection of buds by soil is cryptogeal germination and seeds that germinate at the soil surface but subsequently bury their hypocotyl region through hypocotyl or root contraction (Fisher, 2008). In the southeastern USA, longleaf pine (*Pinus palustris* Mill.) seedlings have a 'grass stage' where the terminal bud remains at the soil surface for several years where it is protected by dense foliage (Bower, 1990).

Shortleaf pine seedlings form a morphological attribute, colloquially identified as a 'basal crook', in which the seedling bends at a point low in the hypocotyl region to lay along the ground. Shortly thereafter, the stem bends upward at a point just above the hypocotyl causing the stem to grow vertically from this point. This process leaves a horizontal basal crook section 2.5–7.5 cm long (Mattoon, 1915; Stone and Stone, 1954) that contains dormant buds that are formed in the axils of the primary needles. Open-grown seedlings develop the basal crook in the first year, but shade-grown seedlings may develop it as late as 3–9 years (Little and Somes, 1956).

The basal crook has been widely speculated to facilitate resprouting by lowering the dormant bud cluster closer to the soil surface where a fire is cooler. In addition, the crook permits the accumulation of soil and duff above the dormant bud cluster, further protecting and insulating the dormant buds. Crooking has been associated with sprouting success following prescribed fire (Little and Somes, 1956). Likewise, the temperature reached at the basal crook during fire was inversely correlated to shortleaf pine resprouting, and resprouting does not occur on charred stem segments (Lilly et al., 2012a). While intuitive, the importance of the basal crook to resprouting following fire has never been directly tested, likely because nearly all naturally regenerated shortleaf pines have basal crooks (Mattoon, 1915; Stone and Stone, 1954; Lilly et al., 2012a) which makes disentangling resprouting potential and the presence of the crook difficult. When topclipped, all shortleaf pine seedlings resprouted (Lilly et al., 2012b). However, resprouting success of seedlings topkilled by fire is variable (Little and Somes, 1956; Lilly et al., 2012a).

While there has been discussion as to the evolutionary pressures that caused resprouting ability to arise (Bradshaw et al., 2011a, 2011b; Keeley et al., 2011), differences in postfire resprouting capacity can determine species composition and ecosystem structure and function. In fire inclusive systems, resprouting ability provides shortleaf pine with a competitive advantage to species that do not normally resprout, such as the closely related loblolly pine (Williams, 1998). Stewart et al. (2015) found that biennially prescribed fires eliminated loblolly pine and most shortleaf pine - × loblolly pine hybrid seedlings while favoring shortleaf pine seedlings, but fire exclusion over 30 years in nearby stands resulted in a mixed seedling population of shortleaf pine, loblolly pine, and their hybrids.

Shortleaf pine × loblolly pine hybrid seedlings are likely killed by fire because they form a crook that is intermediate between a crook with 90° angles found in shortleaf pine and no crook found in loblolly pine (Lilly et al., 2012b; Will et al., 2013). The intermediate basal crook has approximately 45° angles where the dormant buds along the hypocotyl region of the hybrid pines are positioned higher above the soil surface (Will et al., 2013) where they might be exposed to fire, resulting in their elimination when a regular fire regime is employed (Stewart et al., 2015). Determining the role of the basal crook has taken on importance in light of the threat of hybridization between shortleaf pine and loblolly pine. Compared to shortleaf pine, loblolly pine has lower sprouting capacity which is typically lost within three years (Little and Somes, 1956; Campbell, 1985; Lilly et al., 2012b) and does not possess a basal crook. Thus, loblolly pine seedlings generally do not resprout following topkill from fire. Shortleaf pine × loblolly pine F1 hybrid seedlings exhibit rapid height growth like loblolly pine, have resprouting capacity intermediate to parent species following topclipping, and possess an intermediate crook (Will et al., 2013).

Our goal was to determine the effects of fire on resprouting of shortleaf pine, loblolly pine, and their F1 hybrids and relate this to the presence of the basal crook. We planted seeds and then deployed small-scale fires in early spring and summer of the resulting seedlings' second growing season and the spring before their third growing season. Determining the post-fire resprouting success of shortleaf pine, loblolly pine and the F1 hybrid seedlings indicated the role that fire exclusion plays in facilitating persistence of hybrid pine seedling and saplings and the ongoing change in stand composition and population genetics. We also investigated the importance of the basal crook for fire tolerance in shortleaf pine. We manipulated the height of the dormant buds in proximity to the soil for both shortleaf pine and loblolly pine and then measured resprouting after fire. The basal crook's functional role in shortleaf pine fire adaptation may have profound relevance to the ecology and resilience of the forests of the southeastern USA. Understanding that role will be important for forest management and restoration efforts.

## 2. Materials and methods

### 2.1. Seedling origins

Shortleaf pine, loblolly pine, and shortleaf × loblolly hybrid pine seeds were produced at the Oklahoma Forestry Services seed orchard in Idabel, Oklahoma, USA (35°53′N, 94°45′W). Both shortleaf pine and loblolly pine seeds were collected from open-pollinated cones from six shortleaf pines and six loblolly pines originating from the western populations of both species' ranges-- southeastern Oklahoma, southwestern Arkansas, and northeastern Texas. Hybrid pine seeds came from six loblolly pine maternal parents that were control-pollinated with pollen from five shortleaf pine paternal parents. Cones were collected and seeds extracted and stored (−20 °C) using standard protocols.

On 24 January 2013, seeds were removed from storage and soaked in water for 12 h at 4 °C. Seeds that sank were selected for stratification in a refrigerator in a moist paper towel contained in a plastic bag at 4 °C for 56 days. On 28 March 2013, seeds were planted at the Kiamichi Forestry Research Station, Idabel, Oklahoma, USA, which is adjacent to the Oklahoma Forestry Services seed orchard. The field was approximately 0.2 ha and had been tilled prior to planting. The soil was a mixture of Adaton loam (Fine-silty, mixed, active, thermic Typic Endoaqualfs) and Kullit fine sandy loam (Fine-loamy, siliceous, semiactive, thermic Aquic Paleudults), with slope between 0 and 3%.

### 2.2. Comparing hybrid and parent species resprouting

Seeds were planted in 14 rows, each containing 20 evenly-spaced planting positions, in independent random orders of six loblolly pine and six shortleaf pine (one from each half-sib family) as well as eight hybrid pines (one from each full-sib family) resulting in 280 planting positions (20 per row × 14 rows). In each planting position, five to ten seeds were planted by covering with 1–2 cm of mineral soil. Planting positions were marked and labeled by numbered metal tags attached to stakes. Wire enclosures were placed over each planting site and staked into the ground to prevent herbivory.

By 16 days post-planting (13 April 2013), not all planting positions had identifiable seedlings. Additional seeds, which had been cold stratified for 80 days, were planted into 50 mL of prepared soil (three parts field site soil, two parts coarse sand, and one part peat moss) in Leach tubes (Stuewe and Sons, OR, USA) at a greenhouse in Stillwater, Oklahoma on 15 April 2013. These additional seeds were watered and kept at a day-time temperature of 24 °C and a night-time temperature of 20 °C. On 3 May 2013, newly germinated seedlings from the same half- or full-sib families were transplanted into the approximately 100 previously unsuccessful planting positions. These seedlings were planted such that their root collars were at the soil surface after transplanting. Before transplanting, the seedlings were removed from the soil mixture in which they had been growing and planted directly into the soil.

The field was irrigated as needed, May through September of 2013, with the goal of keeping the seedlings alive. Competition control consisted of directed sprays of glyphosate while covering seedlings with a protective canister and hand weeding competition directly adjacent to the seedlings. On 25 June 2013, the planting positions were thinned to leave one to three seedlings such that each seedling had enough space that they would not interfere with one another (>5 cm apart).

On 16 March 2014, one third of the surviving seedlings from each family were randomly selected to be burned. Before burning, seedling height (to the nearest cm), ground line diameter (to the nearest mm), and height to location of primary needles (nearest mm) were measured. Half the rows were randomly selected to receive a layer of duff (1 cm deep layer of moistened needles and leaves that had decomposed on a pine-hardwood forest floor for more than 1 year in a 10 cm diameter circle surrounding the seeding) and the remaining half receiving no duff. In total, 14 of 26 loblolly pines, 14 of 33 hybrid pine, and 10 of 23 shortleaf pine seedlings received duff additions before burning. On 17 and 18 March 2014, selected seedlings were burned between 1100 and 1800 h. Burning the seedlings consisted of piling 1.0–1.4 kg of air-dried loblolly pine litter in a 0.5 $m^2$, approximately 5–7 cm, deep circle around the seedling. Fresh litter was collected in the fall of 2013 and stored in a greenhouse until use.

Before burning, thermocouples (type K) were placed at ground level next to the seedling stem to measure temperature during the fires. The thermocouple wires were attached to a data logger with temperature recorded every 0.5 s during fire, and pine straw was laid over the wires. Fires were started at the downwind edge of the circle with an accelerant and moved through the plots as a backfire, lasting about 6 min. Weather conditions on 17 March 2014 averaged 11.7 °C (ranged from 5.6 to 14.5 °C), relative humidity averaged 58% (ranged from 45% to 77%), and wind speed averaged 2.5 m $s^{-1}$ (ranged from 0.6 to 4.5 m $s^{-1}$). On 18 March 2014, temperatures averaged 19.4 °C (ranged from 15.6 to 22.3 °C), relative humidity averaged 45% (ranged from 40% to 54%), and wind speed averaged 7.8 m $s^{-1}$ (ranged from 5.9 to 9.3 m $s^{-1}$). Weather conditions were measured at the Idabel Weather Station (Oklahoma Mesonet, Oklahoma Climatological Survey). Seedlings were examined for survival and resprouting 43 days later, on 29 April 2014.

A growing season burn was conducted on 25 and 26 August, 2014 on approximately half of the remaining two-thirds of the yet non-burned seedlings were burned (26 loblolly pines, 26 hybrid pines, and 16 shortleaf pines). Ambient temperature during the 25 August 2014 burn averaged 32.4 °C (ranged from 30.5 to 33.8 °C), relative humidity averaged 52% (ranged from 48% to 56%), and wind speed averaged 2.2 m $s^{-1}$ (ranged from 1.5 to 2.8 m $s^{-1}$). On 26 August 2014, ambient temperature averaged 32.9 °C (ranged from 30.3 to 33.9 °C), relative humidity averaged 51% (ranged from 45% to 58%), and wind speed averaged 3.4 m $s^{-1}$ (ranged from 2.1 to 5.6 m $s^{-1}$) (Oklahoma Mesonet, Oklahoma Climatological Survey). The August burn closely followed the methodology of the first burn, but some modifications were made: 1.4 kg of pine straw was added around the seedlings, and duff was not added to any seedlings; location of the primary needles could no longer be determined and was not measured; and seedlings were examined for survival and resprouting 35 days later on 30 September 2014.

A second dormant season burn was conducted on the remaining trees on 16 and 17 March 2015. Ambient air temperature during the 16 March 2015 burn averaged 21.1 °C (ranged from 18.6 to 22.2 °C), relative humidity averaged 64% (ranged from 58% to 76%), and wind speed averaged 3.0 m $s^{-1}$ (ranged from 1.0 to 4.8 m $s^{-1}$). On 17 March 2015, ambient temperature averaged 26.1 °C (ranged from 24.8 to 27.2 °C), relative humidity averaged 53% (ranged from 46% to 60%), and wind speed averaged 2.5 m $s^{-1}$ (ranged from 1.0 to 4.3 m $s^{-1}$) (Oklahoma Mesonet, Oklahoma Climatological Survey). The March 2015 burn closely followed the methodology of the previous burns. Seedlings were examined for survival and resprouting 55 days later on 12 May 2015.

Thermocouple data was screened for missing and erroneous data points. Maximum temperatures were calculated for each planting site burn. The time above 60 °C also was calculated as a measure of duration of exposure above the temperature sufficient to kill the cambium. Proc GLM was used to test for differences in fire temperatures among species in SAS 9.3 and to provide Fisher's exact tests for testing the significance of resprouting among genotypes within each burning date.

### 2.3. Importance of basal crook to resprouting

Seeds from the loblolly pine and shortleaf pine half-sib families discussed above were treated and planted in the same manner and at same time (28 March 2013) in 72 positions in an area adjacent to the hybrid study. On 18 March 2014, seedlings were treated either with top clipping or by burning. Burning was conducted in the same manner as described above. Of the 72 planting positions, 31 had healthy, live seedlings at time of treatment.

Shortleaf pine had three treatments applied: Burned, Exposed, and Clipped. The Burned treatment involved burning a non-manipulated seedling. Non-manipulated shortleaf pine seedlings all had basal crooks that pressed the dormant buds against the ground. The Exposed treatment included a fire treatment like the Burned treatment. However, the soil under the basal crooks was excavated, and a small amount of pine straw was place under the basal crook to expose the previously protected buds to fire. This treatment elevated the dormant buds approximately 5 mm above the soil surface but did not expose any roots. In the Clipped treatment, stems were cut 1–3 cm above the dormant buds and crooks were left undisturbed and not burned. Shortleaf pine had 4 positions (7 seedlings) that were Burned, 6 positions (10 seedlings) that were Exposed, and 4 positions (4 seedlings) that were Clipped.

Loblolly pine also had three treatments applied: Burned, Protected, and Clipped. The Clipped treatment was the same as for shortleaf pine. The Burned treatment involved burning the non-manipulated seedling in the same manner as the shortleaf pine Burned seedlings. In the Burned treatment, loblolly pine dormant buds were naturally exposed to the fire, because loblolly pine lacks a basal crook. To simulate the basal crook's protection of buds from fire, protected loblolly pine had mineral soil mounded up against the stem, covering the dormant buds with a layer of soil 2–4 cm above the height of the dormant buds before burning (Fig. 1). The soil was removed within an hour after fire. Loblolly pine had 5 positions (9 seedlings) that were Burned, 6 positions

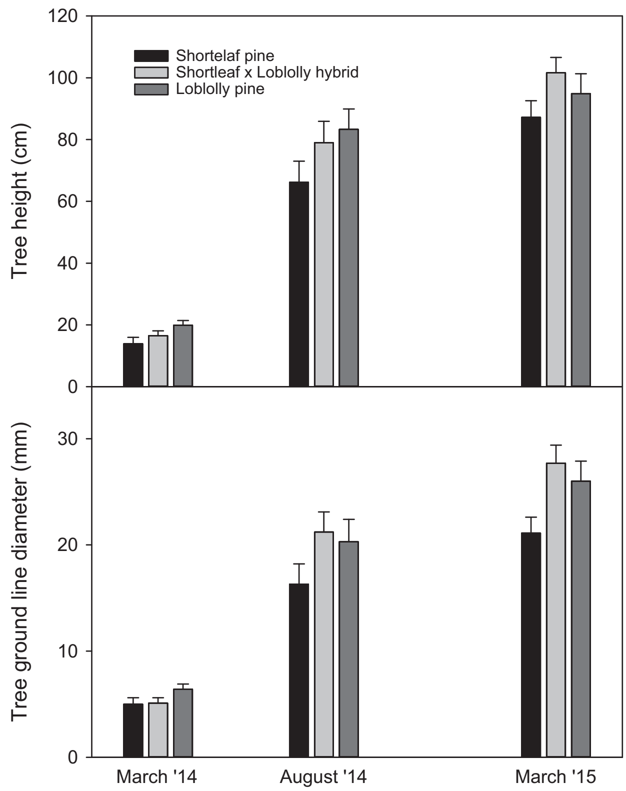


**Fig. 1.** Tree height and ground line diameter for shortleaf pine, shortleaf pine × loblolly pine hybrids, and loblolly pine after one growing season (March 2014), the middle the second growing season (August 2014), and after two growing seasons (March 2015). Vertical bars indicate standard errors.

(13 seedlings) that were Protected, and 6 positions (11 seedlings) that were Clipped.

Ambient air temperature averaged 23.6 °C (ranged from 23.1 to 23.9 °C), relative humidity averaged 36% (ranged from 33% to 38%), and wind speed averaged 6.9 m $s^{-1}$ (ranged from 4.7 to 8.2 m $s^{-1}$) (Oklahoma Mesonet, Oklahoma Climatological Survey). At 37 days post-treatment (24 April 2014) the seedlings were examined for resprouting. For each seedling, both the resprouting status and the number of resprouts were recorded.

Proc Mixed was used to test for differences in number of sprouts per live seedling as well as differences in maximum temperature measured at the soil surface during the fires. Fisher's exact test was used to test for differences in respouting among treatments. The two species were tested separately to determine (1) how exposing the otherwise protected shortleaf pine basal crook affects resprouting and (2) how protecting the otherwise exposed dormant buds of loblolly affects resprouting.

## 3. Results

### *3.1. Comparing hybrid and parent species resprouting*

#### *3.1.1. March 2014 Burn*

In March, before the beginning of the second growing season, the average seedling height and ground line diameter (GLD) were, respectively, 169 mm and 5.5 mm. Loblolly pine seedlings were marginally taller ($p = 0.07$) than shortleaf pine seedlings (Fig. 1). Differences among GLD were not significant ($p = 0.11$). Regardless of seedling size, the fires topkilled all treated seedlings. Only a few of the largest seedlings escaped complete crown scorch, and the foliage of those trees eventually turned brown and died.

Overall (both the duff-added and no-duff treatments combined), 11 of 22 shortleaf pine seedlings burned in March resprouted. In contrast, all 26 burned loblolly pine and 33 of 34 hybrid pine seedlings did not resprout. All but two of the shortleaf pine seedlings had strong basal crooks. No hybrid pine seedlings had strong basal crooks, and the majority (67%) had weak crooks (Fig. 2). The one hybrid pine seedling that resprouted did not have an identifiable crook. Only four of the 26 loblolly pine seedlings had a morphological feature that was scored as a weak basal crook (15%). The remainder (22 seedlings) had no crooks. Resprouting was significantly greater for shortleaf pine in both the case of no duff added ($p = 0.008$) or duff added ($p < 0.0001$) (Fig. 2). Only shortleaf pine seedlings had enough resprouting seedlings to compare the effect of duff-added and the no duff treatments. Duff presence increased shortleaf pine seedling resprouting ($p = 0.09$) with 70% resprouting with duff-added and 33% resprouting without duff treatment (Fig. 2). Height to the primary needles (region of dormant bud location) differed among species. Primary needles were closer to the soil surface in shortleaf pine (8 ± 2 SE mm) than in hybrid pine (20 ± 1 SE mm) ($p < 0.001$), and hybrid pine had lower primary needles than loblolly pine (35 ± 2 SE mm)

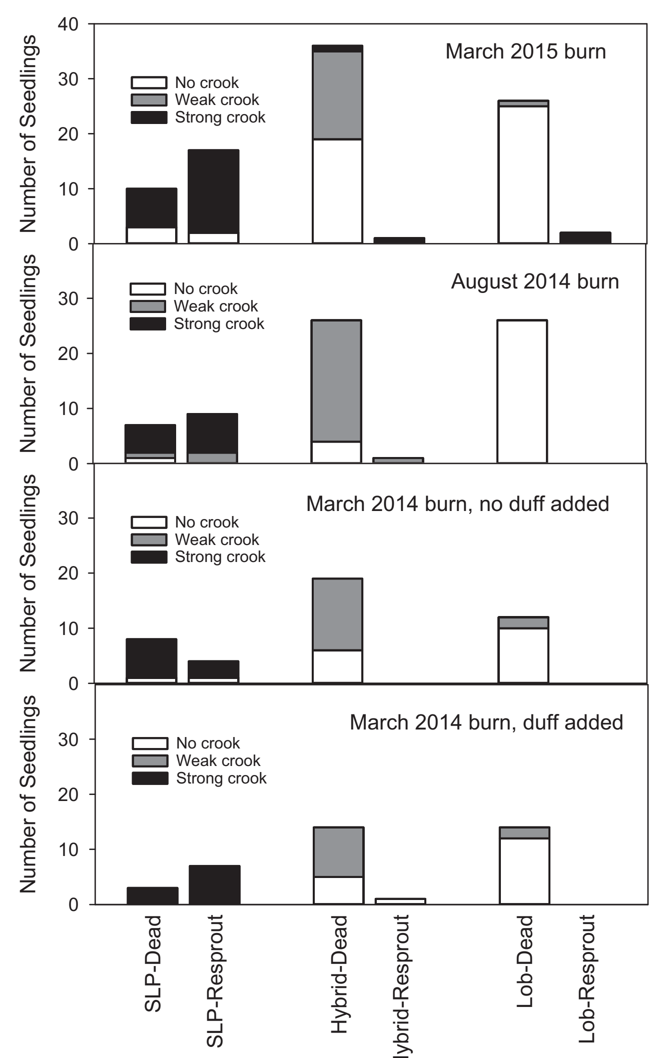


**Fig. 2.** Crook morphology and number of trees that resprouted after topkill from fire for shortleaf pine, shortleaf pine × loblolly pine hybrids, and loblolly pine after one growing season (March 2014), the middle the second growing season (August 2014), and after two growing seasons (March 2015). For the March 2014 burn only, duff was added to approximately half the seedlings before burning.

($p < 0.001$). The sole resprouting hybrid pine's primary needles were 12 mm above the soil.

March burns included the duff or no duff treatment. Within those treatments, fire temperatures at the soil surface were higher in plots treated without duff (424 °C ± 41 SE) than in plots with duff (180 °C ± 38 SE) ($p < 0.0001$). There were not significant differences in temperatures among species ($p = 0.19$). The average maximum temperature was 304 °C ± 35 SE. As a measure of fire duration, the length of time of temperature above 60 °C did not differ among species ($p = 0.14$) and averaged 146 s ± 11 SE.

#### 3.1.2. August 2014 Burn

Towards the end of the second growing season in August, the average seedling height and GLD were, respectively, 783 mm and 21.2 mm. Species effects on height and GLD were not significant ($p = 0.23$, $p = 0.19$, respectively) (Fig. 1). Regardless of seedling size, the growing-season (August) fires topkilled all treated seedlings. Only a few of the largest seedlings escaped complete crown scorch. The trees with incomplete crown scorch had completely brown foliage a month after burn. Of the 16 shortleaf pine seedlings selected to burn, one had no identifiable basal crook (6%), three had weak basal crooks (19%), and 12 had strong basal crooks (75%) (Fig. 2). Of the 27 hybrid pine seedlings, 4 had no basal crook (15%), 23 had a weak crook (85%), and 0 seedlings had strong basal crooks. All 26 loblolly pine seedlings had no basal crook. After topkill, nine shortleaf pine seedlings resprouted (56%), two with weak basal crooks and seven with strong basal crooks. The single resprouting hybrid pine had a weak basal crook. No loblolly pine seedlings resprouted. Occurrence of resprouting among species was significant ($p < 0.0001$). The maximum temperatures for August burns were hotter for shortleaf pine seedlings (550 °C ± 37 SE) than for hybrid pine seedlings (327 °C ± 63 SE) while the loblolly pine seedling temperatures were intermediate (488 °C ± 63 SE) (p = 0.04). Time above 60 °C did not significantly differ among species ($p = 0.30$) and averaged 245 s ± 22 SE.

#### 3.1.3. March 2015 Burn

After two full growing seasons, GLD of loblolly and hybrid pines was greater than shortleaf pine ($p = 0.03$) while height differences were not statistically different ($p = 0.20$) (Fig. 1). For this third burn (a second dormant season burn), one loblolly pine sapling escaped topkill and was excluded from the analysis. Resprouting was significantly greater for shortleaf pine than the other species ($p < 0.0001$). Seventeen of 27 (63%) of shortleaf pine saplings resprouted, and 81% of shortleaf pine possessed a strong basal crook. Two of the resprouting shortleaf pine saplings did not have an identifiable basal crook. One hybrid pine sapling out of 37 seedlings resprouted (3%) and the seedling that resprouted possessed a strong crook. In addition, ants had mounded soil around the lower stem of this sapling. Two loblolly pine saplings out of 28 (7%) resprouted. Both of the resprouting loblolly pine saplings had strong basal crooks, while all that died had no crook (25 saplings) or a weak crook (1 sapling). Maximum temperatures did not differ among species ($p = 0.59$) and averaged 549 °C ± 35 SE. Likewise, time above 60 °C did not differ among species ($p = 0.35$) averaging 394 s ± 34 SE.

### 3.2. Importance of basal crook to resprouting

At time of burn in March 2014, loblolly pine seedlings (25.8 cm ± 2.2 SE) were taller than shortleaf pine seedlings (19.5 cm ± 3.0 SE) ($p = 0.09$). Likewise, GLD of loblolly pine seedlings (8.4 mm ± 0.6 SE) was greater than shortleaf pine seedlings (6.4 mm ± 0.8 SE) ($p = 0.06$). There were no differences in maximum fire intensity between treatments ($p = 0.14$). Maximum fire temperatures near ground level averaged 339 °C ± 28 °C SE. The length of time measured above 60 °C averaged 205 s ± 28 SE seconds.

**Table 1**
Resprouting success of one-year-old shortleaf and loblolly pine seedlings following topkill. Resprouts are the number of sprouts per living seedling. Clipped consisted of cutting the seedling top off above dormant buds. Burned consisted of burning unaltered seedlings. Protected consisted of burning loblolly pine seedlings whose dormant buds were protected by mounding mineral soil. Exposed consisted of burning shortleaf pine whose basal crooks were exposed. NA is not applicable.

| Species | Treatment | Number | Surviving | Percent (%) | Resprouts (SE) |
|---|---|---|---|---|---|
| Loblolly | Clipped | 11 | 11 | 100 | 3.89 (±0.82) |
| | Burned | 9 | 0 | 0 | |
| | Protected | 13 | 6 | 46 | 4.71 (±1.08) |
| | Exposed | NA | | | |
| Shortleaf | Clipped | 4 | 4 | 100 | 7.75 (±0.63) |
| | Burned | 7 | 5 | 71 | 6.00 (±1.41) |
| | Protected | NA | | | |
| | Exposed | 9 | 0 | 0 | |

In the Burned treatment, 71% of shortleaf pine resprouted, and no loblolly pine seedlings survived (Table 1). Exposed shortleaf pine did not resprout when burned, which was significantly lower than the occurrence of resprouting for normal (not exposed) shortleaf pine seedlings that were burned ($p = 0.005$). Forty-six percent of Protected treatment loblolly pine seedlings resprouted following fire, significantly greater survival than that of Burned loblolly pine trees without protection ($p = 0.02$). In the Clipped treatment, 100% of loblolly and 100% of shortleaf pine seedlings resprouted after coppice. Comparing just the seedlings that resprouted, shortleaf pine had more sprouts (6.8 sprouts per surviving seedling) than did loblolly pine (4.2 sprouts per surviving seedling) ($p = 0.02$).

## 4. Discussion

In shortleaf pine, resprouting after topkill depends on how exposed the dormant buds are to fire and the fire intensity. All of the shortleaf pine seedlings with basal crooks artificially exposed to fire died. The resprouting success of shortleaf pine depends on fire intensity (Lilly et al., 2012a). We were unable to evaluate fire intensity on resprouting success because of the general uniformity of fire treatment; the same fuel load and burn conditions were used across replicates. We showed that resprouting depended on whether the basal crook was covered by soil, lay directly on the soil surface, or extended above the soil surface. We noted several instances in which shortleaf pine basal crooks were above the soil surface due to erosion: these trees did not resprout. While the inclusion of a potentially protective duff layer in the first burn (March 2014) did increase shortleaf pine survival, it was not necessary for resprouting. Shortleaf pine produced more sprouts than did loblolly pine when topclipped after one growing season. These results were similar to Lilly et al. (2012b) and Will et al. (2013) and indicate that shortleaf pine has the benefit of greater resprouting capacity as well as protection of buds afforded by the basal crook.

While the basal crook of shortleaf pine is not essential for resprouting, it clearly facilitates resprouting by increasing the likelihood of protection from fire by lowering the height of buds that originally develop aboveground. The basal crook in shortleaf pine is likely an adaptation to the historical frequent fire return interval on drier sites across the southeastern USA where shortleaf pine previously dominated. This trait is analogous to other traits demonstrated by species found in fire-prone environments (reviewed by Clarke et al., 2013). A recent study of 63 tree species across three biomes in South Africa found that increased fire frequency selects for species that either protect buds beneath thick bark or resprout from root systems protected by the soil (Charles-Dominique et al., 2015). Shortleaf pine exhibits the basal crook trait as adaptation to protect buds in a fire-prone environment and facilitate resprouting after fire.

In contrast to shortleaf pine, loblolly pine lacks a basal crook and does not resprout following topkill from fire. When protected by mounding of mineral soil, however, about half resprouted, demonstrating the capacity to resprout when the dormant buds are protected. Compared to the Clipped treatment, in which all loblolly pine seedlings resprouted, some mortality in the Protected treatment may have occurred because the tops were not removed. Will et al. (2013) reported lower survival in loblolly pine seedlings that were girdled with a propane torch above the dormant buds compared to top clipping. They speculated that leaving an intact top in the girdling treatment may have reduced sprouting due to desiccation as foliage above the girdle might still lose water. The foliage of some loblolly pine seedlings in the Protected treatment were not completely incinerated by the fire, leaving scorched needles above the charred branches and stem that could have drawn water away from the buds.

Given that exposure of buds to fire limits resprouting success, resprouting of shortleaf pine × loblolly pine hybrid seedlings should be lower than that for shortleaf pine. Measurements conducted before the first burn indicated that the typical weak crook of the hybrid pines leave the dormant buds 2 cm about the soil on average. In contrast, the dormant buds for shortleaf pine were near or directly on the soil surface. Accordingly, only 3 of 101 hybrid pines resprouted, one of which had a strong crook. Similarly, loblolly pine, which has the greatest height to dormant buds, also did not resprout except for 2 out of 80 trees. Both of the loblolly pine seedlings that resprouted had strong crooks, the only two loblolly pine thus identified, which may indicate an error in planting or an environmental impact that caused the deformation. In either case, this evidence reinforces the importance of bud protection for resprouting after topkill.

With the exception of the August 2014 burn, there were not differences in fire intensity among species that could have contributed to different rates of resprouting among species or treatments. In that case of the August 2014 burn, the maximum temperature was lower for the hybrid seedlings for which only one seedling resprouted. For the first burn, the addition of duff was associated with lower maximum temperatures and could have contributed to greater resprouting of the shortleaf pine than the no-duff treatment. However, adding 1 cm of moist duff did not facilitate survival in either loblolly pine or hybrid pine seedlings, as the moist duff was insufficient to protect the more highly exposed buds.

## 5. Conclusions

Fire exclusion removes an important selection pressure and clearly allows loblolly pine and shortleaf pine × loblolly pine hybrids to persist on sites where they previously would not have survived. Stewart et al. (2015) found that biennial prescribed fires eliminated loblolly pine and most shortleaf pine × loblolly pine hybrid pine seedlings under a mixed canopy of loblolly pine and shortleaf pine in northern Florida, USA. In comparison, fire exclusion in adjacent, non-burned areas allowed loblolly pine and hybrid pine seedlings to persist. The results of our current manipulative study confirm that fire can eliminate loblolly pine and shortleaf × loblolly pine hybrids and thus favor shortleaf pine. Therefore, restoration of shortleaf pine ecosystems, such as those undertaken by the U.S. Forest Service, needs to include frequent fire to eliminate loblolly pine and shortleaf pine × loblolly pine hybrid seedlings. The basal crook also is an important feature to consider when planting nursery-grown seedlings. Seedlings with crooks should be planted with the crook at the soil surface. Those seedlings that come from the nursery without crooks should be planted deeper so that the dormant buds are at or just below the soil surface. This will allow prescribed fire to be used as a tool to select for shortleaf pine via resprouting should natural regeneration of loblolly pine or shortleaf × loblolly pine hybrids become an issue in newly planted shortleaf pine stands.

The basal crook of shortleaf pine, and the fire tolerance that it confers, makes it a crucial trait for the resiliency of the forests of the southeastern USA, which are exposed to a multitude of stresses, including wildfire, which may increase in frequency and intensity due to climate change (Flannigan et al., 2000; Nowacki and Abrams, 2008; Liu et al., 2010). In addition to greater resprouting after topkill from fire, shortleaf pine is more drought, cold, and ice tolerant than loblolly pine. Forests with a pure shortleaf pine population are more likely to persist if disturbance from wildfire and droughts increases in the future as shortleaf pine will better endure than loblolly pine or shortleaf pine × loblolly pine hybrids (Stewart et al., 2015).

## Acknowledgements

We would like to thank the Oklahoma Agricultural Experimental Station, McIntire-Stennis project OKL0 2929, and USDA Forest Service Cooperative Agreement 14-CA-11330126-104 for providing funding for this research. We are deeply grateful for the assistance from Bob Heinemann and the staff at the Kiamichi Forestry Research Center, including Cassandra Meek, Randy Holeman, Dennis Wilson, Glenda Robinson, and Keith Anderson. We would also like to thank Professor Emeritus Charles Tauer for making the shortleaf × loblolly crosses and initiating this line of research. We also appreciated the help of Duncan Wilson, Adam Maggard, Dipesh KC, Lays Francisco, Rodrigo Carvalhais, and Casey Ausmus at various crucial points in this research.

## References

Bower, W.D., 1990. Longleaf pine. In: Burns, R.M., Honkala, B.H. (Eds.) Silvics of North America. Volume I. Conifers. Handbook 654. USDA Forest Service, Washington, DC, pp. 405–412.

Bradshaw, S.D., Dixon, K.W., Hopper, S.D., Lambers, H., Turner, S.R., 2011a. Little evidence for fire-adapted plant traits in Mediterranean climate region. Trends Plant Sci. 16, 69–76.

Bradshaw, S.D., Dixon, K.W., Hopper, S.D., Lambers, H., Turner, S.R., 2011b. Response to Keeley et al.: fire as an evolutionary pressure shaping plant traits. Trends Plant Sci. 16, 405.

Campbell, T.A., 1985. Sprouting of Slash, Loblolly, and Shortleaf Pines Following a Simulated Precommercial Thinning. Forest Service Note SO-320. USDA Forest Service, New Orleans, LA, p. 3.

Charles-Dominique, T., Beckett, H., Midgley, G.F., Bond, W.J., 2015. Bud protection: a key trait for species sorting in a forest-savanna mosaic. New Phytol. 207, 1052–1060.

Clarke, J.J., Lawes, M.J., Midgley, J.J., Lamont, B.B., Ojeda, F., Burrows, G.E., Enright, N. J., 2013. Resprouting as a key functional trait: how buds, protection and resources drive persistence after fire. New Phytol. 197, 19–35.

Flannigan, M.D., Stocks, B.J., Wotton, B.M., 2000. Climate change and forest fires. Sci. Total Environ. 262, 221–229.

Fisher, J.B., 2008. Anatomy of axis contraction in seedling from a fire prone habitat. Am. J. Bot. 95, 1337–1348.

Guldin, J.M., 1986. Ecology of shortleaf pine. In: Murphy, P.A. (Ed.), Proceedings of the Symposium on the Shortleaf Pine Ecosystem. Arkansas Cooperative Extension Service, Monticello, AR, pp. 25–40.

Keeley, J.E., Pausas, J.G., Rundel, P.W., Bond, W.J., Bradstock, R.A., 2011. Fire as an evolutionary pressure shaping plant traits. Trends Plant Sci. 16, 406–411.

Lilly, C.J., Will, R.E., Tauer, C.J., Guldin, J.M., Spetich, M.A., 2012a. Factors affecting sprouting of shortleaf pine rootstock following prescribed fire. For. Ecol. Manage. 265, 13–19.

Lilly, C.J., Will, R.E., Tauer, C.G., 2012b. Physiological and morphological attributes of shortleaf × loblolly pine F1 hybrid seedlings: is there an advantage to being a hybrid? Can. J. For. Res. 42, 238–246.

Little, S., Somes, H.A., 1956. Buds enable pitch and shortleaf pines to recover from injury. Station Paper no. 81. USDA Forest Service. Upper Darby, PA.

Liu, Y., Stanturf, J., Goodrick, S., 2010. Trends in global wildfire potential in a changing climate. For. Ecol. Manage. 259, 685–697.

Mattoon, W.R., 1915. Life history of shortleaf pine. Bulletin No. 244. US Department of Agriculture. Washington, DC.

McWilliams, W.H., Sheffield, R.M., Hansen, M.H., Birch, T.W., 1986. The shortleaf resource. In: Murphy, P.A. (Ed.), Proceedings of the Symposium on the Shortleaf Pine Ecosystem. Arkansas Cooperative Extension Service, Monticello, AR, pp. 9–24.

Nowacki, G.J., Abrams, M.D., 2008. The demise of fire and "mesophication" of forests in the eastern United States. Bioscience 58, 123–138.

Oswalt, C.M., 2011. Spatial and temporal trends of the shortleaf pine resource in the eastern United States. In: Kush, J.S., Barlow, R.J., Gilbert, J.S. (Eds.), Proceedings of the shortleaf pine conference: East meets west. Alabama Agricultural Experiment Station, Special report no. 11. Auburn, AL, pp. 33–36.

Pausas, J.G., Keeley, J.E., 2014. Evolutionary ecology of resprouting and seeding in fire-prone ecosystems. New Phytol. 204, 55–65.

Pausas, J.G., Pratt, R.B., Keeley, J.E., Jacobsen, A.L., Ramirez, A.R., Vilagrosa, A., Paula, S., Kaneakua-Pia, I.N., Davis, S.D., 2016. Towards understanding resprouting at the global scale. New Phytol. 209, 945–954.

Stewart, J., Tauer, C., Nelson, C.D., 2012. Bidirectional introgression between loblolly pine (*Pinus taeda* L.) and shortleaf pine (*P. echinata* Mill.) has increased since the 1950s. Tree Genet. Gen. 8, 725–735.

Stewart, J.F., Will, R.E., Robinson, K.M., Nelson, C.D., 2015. Frequent fire protects shortleaf pine (*Pinus echinata*) from introgression by loblolly pine (*P. taeda*). Conserv. Genet. 16, 491–495.

Stone Jr., E.L., Stone, M.H., 1954. Root collar sprouts in pine. J. For. 52, 487–491.

Tauer, C.G., Stewart, J.F., Will, R., Lilly, C., Guldin, J., Nelson, C.D., 2012. Hybridization leads to loss of genetic stability in shortleaf pine: unexpected consequences of pine management and fire suppression. J. For. 110, 216–224.

Will, R., Lilly, C., Stewart, J., Huff, S., Tauer, C.G., 2013. Recovery from topkill of shortleaf pine × loblolly pine hybrids compared to their parent populations. Trees-Struct. Funct. 27, 1167–1174.

Williams, R.A., 1998. Effects of fire on shortleaf and lobolly pine reproduction and its potential use in shortleaf/oak/hickory ecosystem restoration. In: Waldrop, T. A. (Ed.). Proceedings of the 9th Biennial Southern Silviculture Research Conference. General Technical Report SRS-20. USDA Forest Service. Washington, DC, pp. 321–325.