

THE RESPONSE OF SHORTLEAF X LOBLOLLY  
HYBRID PINE SEEDLINGS TO WATER STRESS AND  
FIRE: IS LACK OF DISTURBANCE ALLOWING  
HYBRIDS TO DISPLACE SHORTLEAF PINE?

By

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**Title of Study:** THE RESPONSE OF SHORTLEAF X LOBLOLLY HYBRID PINES  
TO WATER STRESS AND FIRE: IS LACK OF DISTURBANCE ALLOWING  
HYBRIDS TO DISPLACE SHORTLEAF PINE?

**Major Field:** Natural Resources Ecology and Management

**Abstract:** Shortleaf pine (*Pinus echinata* Mill.) is a hardy pine species. It possesses the widest range of any southern pine and once covered millions of hectares of the southern United States. Loblolly pine (*Pinus taeda* L.) is the most commercially important species in the south with over a billion seedlings planted annually. Logging, land use change, fire exclusion, and other factors have led to a significant decline of shortleaf pine across much of its former range. Among the threats to shortleaf pine is a sudden increase in the number of shortleaf x loblolly hybrid pines in the seedling cohort. The presence of a hybrid swarm is alarming because the hybrid pine can a) outgrow shortleaf pine and b) interbreed with shortleaf pines, threatening shortleaf pine's genetic integrity. While shortleaf and loblolly pines are well studied, the characteristics of the hybrid pine are less known. The traits that may be responsible for the hybrid pine's sudden increase in the last 60 years are particularly interesting. I tested the importance of the basal crook to shortleaf pine's fire tolerance. I also tested shortleaf, hybrid pine, and loblolly pine seedlings' response to water stress by measuring allometry and transpiration under well watered and water stressed conditions. Finally, I tested the shortleaf, hybrid, and loblolly pine seedlings response to fire. The basal crook (which the hybrid pine lacks) was shown to be a crucial morphological feature - shortleaf cannot resprout after fire without it. Shortleaf, hybrid pine, and loblolly pine seedlings were similar in most allometric measurements. However, shortleaf pine possessed greater coarse root biomass and loblolly pine possessed greater stem biomass. I did not find any differences in regulation of transpiration between the three species. In the fire studies, shortleaf pine seedlings were far more successful at resprouting after fire than hybrid pine or loblolly pine seedlings. These results lead us to conclude that the rapid increase in the numbers of hybrid pine is likely due to the exclusion of fire. Reintroducing fire will reduce the number of hybrid pines seedlings and restore shortleaf pine.

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## CHAPTER I

### Introduction

Shortleaf pine (*Pinus echinata* Mill.) was once the dominant southern pine in the United States, it possesses a range that included 24 states. The extensive range was due, in part, to shortleaf pine's resilience. It is able to tolerate ice, wind, extreme temperatures, drought, and fire. This tolerance led to shortleaf pine historically existing in pure stands in recently disturbed areas. In areas not recently disturbed, shortleaf pine would persist in the overstory but not in the seedling cohort, as shortleaf pine prefers full light. Through a variety of factors, including logging, habitat fragmentation, land use change, climate change, and fire exclusion, shortleaf pine now occupies a much smaller space than it once did. Further threatening the shortleaf pine population, recent research has shown an increasing shortleaf x loblolly hybrid swarm which imperils the genetic and phenotypic purity of the shortleaf pine population. This hybrid pine must be better studied in order to understand and mitigate the threat which introgression poses to shortleaf pine forests. Specifically, testing the hybrid pine's tolerance to drought and fire disturbance will provide land managers with strategies to remove the hybrid pine from the seedling cohort and allow us to predict how an increasingly hybrid pine forest will respond to these two frequent disturbances.

Previous work at Oklahoma State highlighted the rise of a hybrid pine swarm (Stewart et al., 2012). In addition, some light has been shed on traits of the hybrid pine. Lilly et al. (2012) found that shortleaf x loblolly pine seedlings were intermediate in many traits, including the basal crook which is an important morphological trait that enables shortleaf pine seedlings to resprout after fire. Loblolly pine (*Pinus taeda* L.) lacks a basal crook. Lilly et al. also found that hybrid pine seedlings may have higher instantaneous water use efficiency than loblolly pine seedlings, but did not impose water stress on the seedlings. Similarly, Will et al. (2013) tested an important fire tolerance characteristic, resprouting ability, of the hybrid pine by using a propane torch to girdle the seedlings. This could not evaluate the effect of the basal crook when estimating the hybrid pine's resprouting ability after normal topkill from fire. While these studies provide valuable and novel insights, their limitations prevent us from fully understanding the reasons for the hybrid pine's sudden surge in numbers, or how the hybrid pine will react when confronted with disturbances in the wild.

My thesis contains three studies. The first tested if the basal crook is an important morphological feature which allows shortleaf pine seedlings to survive fire better than loblolly pine seedlings (which lack the basal crook). The second study examined the allometric responses and transpiration strategies of shortleaf pine, the shortleaf x loblolly hybrid pine, and loblolly pine seedlings. The final study tested the response of shortleaf pine, the hybrid pine, and loblolly pine seedlings to natural topkill by fire.

The shortleaf x loblolly hybrid pine is now threatening the shortleaf pine population through competition and introgression. In this thesis, I aim to better understand the hybrid pine's responses to drought and fire - in doing so, we will have a better understanding of

the factors that have allowed the hybrid pine to succeed in recent years, the implications of increasing hybrid swarm, and how to manage these forests to restore the resiliency of the shortleaf pine forest.

## **CHAPTER II**

### **Literature Review**

#### **I. Introduction to Shortleaf Pine**

##### **Natural History**

Shortleaf pine (*Pinus echinata* Mill.) has the largest natural range of pine in the southern United States, stretching from Florida to Illinois and New Jersey, and from the Atlantic to Oklahoma and Texas (Mattoon, 1915). Shortleaf pine likely once covered a wide area of the south in the form of pure stands but as early as 1915, Mattoon estimated that shortleaf pine had lost 60-80% of its pure type in its range due to logging.

Shortleaf pine is identifiable from other southern pines through several characteristics: needles 7-11 cm in length; 2-3 needles per fascicle; small, egg shaped cones (4-6 cm) with slender needle pointed prickles; reddish brown bark, irregularly plated; a short and narrow crown; and the formation of a basal crook as a seedling (Little, 2002; Mattoon, 1915).

Shortleaf pine is an "ideal" tree for saw timber (Mattoon, 1915), having more desirable form than many other southern pines, including loblolly pine (*Pinus taeda* L.). On good sites, shortleaf pine can reach 25 to 30 meters, and diameters of up to 90 cm (Mattoon, 1915).

The vast majority of pure shortleaf pine forests were logged by European settlers as they moved west. In the north and western portions of its range, shortleaf pine is the most commonly regenerated species (Lawson, 1990), serving as an important tree species ecologically and economically.

## **Reproduction**

Shortleaf pine is monoecious, with flowering occurring from March to April. Shortleaf pine cones ripen from October to November, and seeds will disperse shortly after (Krugman and Jenkinson, 1974). Southern pines are likely pollinated early by near sources (e.g. water drops carrying pollen from male strobili to a female strobilus) and later by other dispersive sources, like wind (Greenwood, 1986). Greenwood (1986) suggested that there is no penalty in fecundity for late pollination, so wind pollination is likely the most common source of pollination. Dyer and Sork (2001) concurred, but with the caveat that pollen is not highly mobile over long distances in shortleaf pine, especially in dense stands. Female strobili are likely fertilized by pollen from neighboring trees, although, the potential does exist for pollen to successfully travel from long distances (Williams, 2010).

Shortleaf pine's seed production is strongly influenced by the climatic variables in the two years preceding seedfall, shortleaf pine will produce greater quantities and quality of seeds if strobili differentiation occurs in wet conditions and enjoys a cool, moist summer (Cain and Shelton, 2000). Occasionally, a seed may be viable two years after its development if it is suspended above the forest floor - however, seeds are not likely to last more than one season after dropping. Shortleaf pine are notoriously variable in their seed crops, ranging from millions of seeds per hectare to none based on the year

(Wittwer and Shelton, 2004). Shortleaf pine seeds are highly likely to fall within 50 meters of the parent tree (Yocum, 1968).

### **Pathogens and Disturbance**

Aside from littleleaf disease (*Phytophthora cinnamomi* Rands), shortleaf pine is a hardy tree, able to tolerate wind, ice, extreme temperatures, and diseases commonly associated with other southern pines (Hepting, 1971; Lawson, 1990; Rink and Wells, 1988). Shortleaf pine is able to establish across a variety of climates, soils, elevations, and topographies but grows best on deep, well drained soils of fine sandy loam, or silty loam textures (Lawson, 1990; Turner, 1935). Shortleaf pine is reported to grow better on drier sites than other southern pines (e.g. loblolly pine), perhaps because of its more massive root system (Lawson, 1990). Lilly et al. (2012a) measured higher instantaneous water use efficiency (lower  $C_i$ ) in shortleaf pine seedlings than in loblolly pine seedlings. Growing in poorly drained soils may put shortleaf pine at a greater risk of succumbing to littleleaf disease (Copeland and McAlpine, 1955).

### **Shortleaf Pine and Fire**

Perhaps the most important physical feature of the shortleaf pine seedlings is the basal crook, which forms early in the seedling's development and persists until the age of about 10, depending on site conditions (Mattoon, 1915). The basal crook is a feature found in young shortleaf pine and also pitch pine (*Pinus rigida* Mill.). The basal crook increases the odds of the seedling resprouting following topkill by fire (Little and Somes, 1956). Specifically in shortleaf pine, the basal crook protects the dormant buds located in the axils of the primary needles from fire by pressing the dormant buds against the soil surface where they are occasionally covered under soil and duff (Stone and Stone, 1954).



If a shortleaf pine seedling is topkilled by fire, the protected dormant buds will resprout prolifically, in as short as 24 hours of topkill (Liu et al., 2011). Eventually, one or two of the resprouts will act as a leader (Mattoon, 1915). Following a mid-intensity fire, Lilly et al. (2012b) found that over half of shortleaf seedlings resprouted in a northwest Arkansas and well over half of those resprouting successfully survived the growing season.

Historic natural disturbances include strong winds, ice storms, droughts, and insects (Bragg, 2003; Turner, 1935). However, the most frequent disturbance was fire (Fowler and Konopik, 2007). Shortleaf pine's ability to resprout as a seedling following topkill from fire is important since the land shortleaf pine occupies (ridges, well drained soils, upland sites) was historically exposed to frequent fire (e.g. Engbring et al., 2008; Guyette et al., 2006; Iniguez et al., 2008; Stambaugh and Guyette, 2006). Stambaugh et al. (2007) found that shortleaf pine abundance decreased in years without fire, suggesting that shortleaf pine is not only fire tolerant, but relies on fire to both establish seedlings and reduce faster growing competition. Yocum and Lawson (1977) found that burning provided more total area suitable for shortleaf pine seed development than areas undisturbed, or areas disturbed by logging.

Shortleaf pine forests may be perpetuated by fire. Williams (1998) found that the majority of regeneration after a Louisiana fire was from resprouting pine seedlings and the vast majority of those resprouts (90-100%) were shortleaf pine seedling - a single fire turned a mixed loblolly-shortleaf pine seedling cohort into a nearly pure shortleaf pine seedling cohort. Shelton and Cain (2002) found during a winter burn, 95% of damaged shortleaf pine resprouted, however, no resprouting was observed during a summer burn

Further support of shortleaf pine's fire adapted quality comes from Stewart et al. (2014) who studied a Florida pine-savanna which was frequently burned. In this savanna, pine seedlings and saplings were made up almost exclusively of shortleaf pine, with a small percentage of shortleaf x loblolly hybrid pine. However, in nearby unburned forests, the seedlings and saplings were composed of shortleaf pine, loblolly pine, and shortleaf x loblolly pine hybrid.

In the more recent past, fire has been actively suppressed, which leads to long periods without fire punctuated by extreme fire events (Fowler and Konopik, 2007). The longer periods without fire allow fast growing, shade tolerant hardwood species to outcompete shortleaf pine seedlings. After establishment, shade tolerant hardwood species will form a midstory which prevents shortleaf pine regeneration (Clewett, 2011). The now shaded understory will be cooler and moister - improving microsite conditions for shade-tolerant species and worsening conditions for fire, and fire-tolerant species (Nowacki and Abrams, 2008). Aside from hardwood species competition, fire exclusion allows faster growing pine (e.g. loblolly pine) to outcompete establishing shortleaf pine seedlings (Lawson, 1990; Schultz, 1997). Even outside its native range, loblolly pine can outgrow shortleaf pine, attaining greater heights and volumes in the relatively dry Oklahoma climate (Dipesh et al., 2015).

## **II. The Rise of Loblolly Pine and the Decline of Shortleaf Pine**

### **Natural History of Loblolly Pine**

Loblolly pine was historically limited to bottom lands and areas not frequented by fire, or wet lands (Schultz, 1999) where they earned their common name by inhabiting swampy areas that reminded Europeans of stew, or "loblolly." The native range of

loblolly pine is 14 states from southern New Jersey south to central Florida and west just into Oklahoma and into eastern Texas (Schultz, 1997). Loblolly pine grows quickly, outpacing shortleaf pine (Cain, 1990; Schultz, 1997; Williston, 1958, 1985). The early years of seedling growth seems to be the time period where loblolly pine outgrows shortleaf pine - the differences in growth rates disappear as the species mature (Williston, 1972).

Presently, loblolly pine is the most important commercial pine in the southern United States, with 12 million acres of loblolly forest and an additional 12 million acres of loblolly plantation (Neale et al., 2004). Schultz (1997) estimated that loblolly pine makes up well over half of all the pine planted in the south while Mckeand et al. (2003) figured that 1.2 billion loblolly pine seedlings are planted annually, composing over 80% of the planted trees in the south. Loblolly pine directly or indirectly contributes \$30 billion to the economy of the southern United States (Schultz, 1999).

Loblolly pine is identifiable by its: long needles (13-22 cm) in bunches of 3-4 per fascicle. Cones are 7.5-15 cm and narrowly conical with stout spines on each scale. Loblolly pine's bark is light to dark brown, turning reddish brown or gray with age - useful in identification; loblolly pine's bark does not display the resin blisters that are common on most shortleaf pine (Baker and Langdon, 1990; Schultz, 1997).

Loblolly pine is monoecious like shortleaf pine. Unlike shortleaf pine, it typically flowers earlier in the season, with pollen shed occurring between February and April, sometimes into March (Grano, 1973; Lawson, 1990). While loblolly pine does not typically share the same flowering time as shortleaf pine, unusual weather can cause loblolly pine pollen shed to occur later, overlapping with shortleaf pine's pollen shed

(Zobel, 1953). When loblolly pine sheds pollen during the time that shortleaf pine is receptive, hybridization is possible between the two species. Loblolly pines are most fecund when seed development is preceded by wet spring weather and dry summers (Grano, 1973).

### **Pathogens and Disturbance**

While shortleaf pine is generally immune to the effects of fusiform rust (*Cronartium quercuum* f. sp. *fusiforme*), loblolly pine can be severely affected by the cankers formed on its stem, and the frequency of infection has increased with the practice of modern silviculture (Snieszko et al., 2014). Eneback et al. (2004) estimate that 10% of southern pines (including loblolly pine) in Alabama have potentially lethal infections of fusiform rust.

If shortleaf pine's decline is partially due to fire suppression (Bragg, 2004), loblolly pine's expansion may be due to the exclusion of fire (Zhang et al., 2010). Williams (1998) found that after a fire under a shortleaf-loblolly pine canopy, 50-85% of pine regeneration was from resprouting pine seedlings, and 90-100% of those resprouting pines were shortleaf pine. Their results highlight both the importance of seedling resprouting following fire and the relative inability of loblolly pine to resprout following fire. Further evidence of loblolly pine seedlings' inability to resprout after topkill as successfully as shortleaf pine seedlings is reported by Liu et al. (2011), who described shortleaf pine seedlings resprouting ability as "extraordinary... Large numbers of sprouts were seen within 2 days after top removal" while characterizing loblolly pine seedlings' lackluster response to topkill as, "only a few sprouts about 1 week following top removal."

### **III. Comparisons of Shortleaf and Loblolly Pines**

#### **Fire**

As long as the dormant buds in the axils of the primary needles are protected, both loblolly and shortleaf pines are capable of resprouting (Shelton and Cain, 2002).

However, sprouting will not occur if a seedling is damaged below the primary needles, where the dormant buds are hidden below the axils of the primary needles. Loblolly pine has a reduced ability to resprout after fire likely because it lacks shortleaf pine's basal crook which is densely packed with dormant buds and protected from damage by being pressed against the ground (Stone and Stone, 1954). Mattoon (1915) praised the basal crook by saying, "the double crook [basal crook] ...characteristic of and always present in young trees, seems to be intimately associated with its power of reproduction by sprouts."

Loblolly pine does not form a basal crook, rather its stem grows perpendicular to the ground, raising the dormant buds far above the placement of shortleaf pines' dormant buds, making the dormant buds more likely to be burned by fire. Cambell (1985) describes loblolly pine as able to resprout after topkill, however, a six inch section of stem above the soils was recommended to be left in order for the seedling to resprout. Without six inches of dormant buds, loblolly pine seedlings were unable to resprout, unlike the shortleaf pine seedlings in the study, which were able to resprout from just two inches of stem left above the ground. Another example of loblolly pine's poor fire tolerance, Wade (1993) found prescribed fire removed young loblolly pine ( $GLD < 1.5$  inches) from overcrowded stands.

#### **Drought**

Shortleaf pine is not just more fire tolerant than loblolly pine, it is anecdotally more drought tolerant than loblolly pine as well (Lawson, 1990; Schultz, 1997). Drought can cause mortality from any of three mechanisms: hydraulic failure, carbon starvation, or biotic agents (McDowell et al., 2008). Drought strategies can be broadly categorized as either isohydric, or anisohydric (e.g. Tardieu and Simonneau, 1998). Isohydric species will close their stomata completely when faced with water stress - this strategy may be effective for short, severe droughts, but it will ultimately face carbon starvation if drought persists. Anisohydric species will continue to transpire at a reduced rate during drought but are more prone to hydraulic failure.

Gonzalez-Benecke and Martin (2010) describe loblolly pine's response to water stress as anisohydric but also isohydrodynamic. Loblolly pine's regulation of transpiration as soil dries means that mid-day water potentials are far higher in control treatments than in irrigated treatments, making it anisohydric. However, loblolly pine's relatively stable difference in water potential from the soil to the needles across treatments was evidence of isohydrodynamicism. Franks et al. (2007), the first to propose the isohydrodynamic strategy, hypothesized that isohydrodynamicism is linked to processes that are sensitive to a gradient of water potential in the plant, rather than any specific water potential threshold. Isohydrodynamicism is a strategy that a plant can use to respond more dynamically to environmental conditions (e.g. soil water potential). Domec et al. (2009) suggested that competing water potentials between loblolly pine roots and needles may be the signal Franks et al. hypothesized necessary for the needles to lower stomatal conductance in order to avoid cavitation in the xylem.

While shortleaf pine is considered more drought tolerant than loblolly pine. Schopmeyer (1939) found shortleaf pine seedlings acted in a more anisohydric manner than loblolly pine seedlings by transpiring greater amounts of water at both high and low soil moistures. Simultaneously, shortleaf pine had higher total water content in its tissues than loblolly pine seedlings. Schopmeyer concluded by saying that shortleaf pine's greater drought tolerance relative to loblolly pine could not be explained by reducing transpiration, nor by forming bound water, nor higher osmotic pressure.

Ting and Chang (1985) measured characteristics frequently associated with drought tolerance between similar stands of loblolly pine, shortleaf pine, and longleaf pine (*Pinus palustris* Mill.) - they found that shortleaf pine had few stomata per mm<sup>2</sup> (114) than loblolly pine (145). They also found that the shortleaf pine stand had a greater amount of water in the soil than did loblolly pine. While the authors failed to record leaf area index of each stand, they did report similar basal areas between shortleaf and loblolly pines (39.48 m<sup>2</sup> per 0.4 ha, 37.85 m<sup>2</sup> per 0.4 ha, respectively). These results would suggest that shortleaf pine is more isohydric than loblolly pine, ostensibly possessing a reduced ability to transpire water (fewer stomata per leaf area) and leaving more water in the soil than loblolly pine stands.

Lilly et al. (2012a) indirectly measured the instantaneous water use efficiency (WUE) of shortleaf pine, loblolly pine, and shortleaf x loblolly hybrid pine seedlings grown in Oklahoma by measuring intracellular CO<sub>2</sub> (*C<sub>i</sub>*) with a LI-COR 6400 Portable Photosynthesis System. They found that shortleaf pine had lower rates of *C<sub>i</sub>* (i.e. instantaneous water use efficiency) than loblolly pine, due to maintaining a higher rate of photosynthesis while having lower stomatal conductance (although neither of those

variables were significantly different from loblolly pine on their own). The explanation of shortleaf pine's drought tolerance is then more isohydric than loblolly pine, limiting transpiration when exposed to water stress.

Lilly et al. (2012a) also measured long term WUE of shortleaf pine and loblolly pine seedlings through  $\delta^{13}\text{C}$  measurements. By examining the ratio of  $^{13}\text{C}$  to  $^{12}\text{C}$  present in shortleaf pine and loblolly pine seedling needle tissue, they were able to publish for the first time that shortleaf pine seedlings do possess higher long term WUE than loblolly pine seedlings.

The conflicting results of Schopmeyer (1939) (anisohydric) and Ting and Chang (1985) and Lilly et al. (2012a) (isohydric) can be reconciled by integrating the work of Macfall et al. (1991). Studying the root system of loblolly pine seedlings grown in sand, they describe, "significant regions of water extraction by the taproot" when using magnetic resonance imaging. Shortleaf pine's large taproot (Lawson, 1990; McQuilken, 1935) may give it an advantage in exploiting more soil moisture relative to loblolly pine. Perhaps a complimentary adaptation, Schopmeyer also found higher solute concentration (and greater osmotic potential) when needle moisture content is the same as loblolly pine. Shortleaf pine's more negative solute potentials, coupled with a more exploitative root system may provide shortleaf pine with a larger soil water volume to transpire, relative to loblolly pine. Schopmeyer conjectured that, "The simultaneous occurrence of a higher total tissue water content and a higher transpiration rate in leaves of shortleaf pine when soil moisture was at the wilting coefficient, indicates that this species had a faster rate of absorption of water from soil than loblolly pine when soil moisture was limited."



## **IV. Shortleaf x Loblolly Hybrid Pines**

### **History of Shortleaf x Loblolly Hybrid Pines**

Shortleaf x loblolly hybrid pines historically occurred infrequently in the areas where shortleaf pine and loblolly pine are sympatric (Stewart et al., 2012). Although not a separate species, for the sake of simplicity and readability, I will refer to shortleaf, hybrid pine, and loblolly pine as "species" in this thesis. Little and Righter (1965) reported that shortleaf x loblolly pine hybrids were made at the Institute of Forest Genetics in Placerville, CA as early as 1933. Zobel (1953) first hypothesized that a wild hybrid may exist between shortleaf and loblolly pines when he came across a tree with the form of a shortleaf pine growing in a low, moist site. Zobel went on to describe confusingly intermediate bark, cones, and seeds from this specific tree. He also mentions that punitive shortleaf x loblolly hybrid seed had been collected as early as 1929, and sent out to be planted across the United States. The only known offspring was an anemic sapling with loblolly pine characteristics. When artificially produced, the shortleaf x loblolly hybrid is usually produced from shortleaf pine pollen and loblolly pine cones (e.g. La Farge and Hunt, 1980).

Apparently, Zobel was unaware of the work of the Gaylord Container Corporation, from Louisiana (Henry and Bercaw, 1956). Shortleaf x loblolly pine hybrid seed was sown in a field of slash pine (*Pinus elliottii* Engelm.) along with a few accidentally introduced loblolly pine. After five years, the shortleaf x loblolly hybrid seedlings were free of fusiform rust, while well over half of the 97 slash pine and all three loblolly pines examined had fusiform cankers. Henry and Bercaw concluded by saying

that future research was planned at the Southern Institute of Forest Genetics. Developing resistance to fusiform rust was the motive for much of the future work on hybrid pine.

Mergen et al. (1965) took Zobel's 1953 conjecture as impetus to study putative hybrids in the sympatric range in Mississippi. They found that although there was great variation between sites, hybrid pine could be distinguished from their parent species by a collection of morphological measurements - the most distinguishing of which was the length of the male strobili. Mergen et al. were the first to state that the hybrid pine had vegetative characteristics similar to loblolly pine and reproductive characteristics similar to shortleaf pine.

### **Pathogen Resistance in Shortleaf x Loblolly Hybrid Pine**

Much of the impetus to study the shortleaf x loblolly hybrid pine has been for the sake of producing fusiform rust resistant pines with the fast growth qualities of loblolly pine. Hare (1969) suggested that introgression between shortleaf and loblolly pines may explain the resistance of western loblolly pine populations to fusiform rust. Hare was the first to suggest that introgression was more common in the western populations of loblolly pine (a hypothesis supported by later work). Both F1 and F2 hybrid pine are immune to fusiform rust, according to Sluder (1970), potentially explaining the development of fusiform resistant loblolly pine populations in the west - repeatedly backcrossed hybrids may have provided the western loblolly pine population with a degree of immunity to fusiform. Schmidtling et al. (2005) acknowledged the potential for hybridization to have provided the initial genetic variation to have formed resistance to fusiform rust in loblolly pine populations west of the Mississippi. However, they concluded that hybridization was not the proximate cause of fusiform resistance in

western loblolly pine by examining alloenzymes and cortical monoterpenes. They attribute the development of resistance to selection pressure, not hybridization.

Hybrid pine has growth rates similar to loblolly pine while having increased resistance to littleleaf disease like a loblolly pine (Schoenike et al., 1977) and immunity to fusiform rust like a shortleaf pine (La Farge and Kraus, 1977). The hybrid pine's increased rate of growth (occasionally growing even faster than loblolly pine) and pathogen resistance made it of particular interest to silviculturalists (e.g. Benson et al., 1982; La Farge and Kraus, 1980). Kraus (1986) published an article encouraging breeders to start their own hybrid pine lines to develop rust resistant, fast growing pine.

### **Identifying Wild Hybrids**

Identifying wild hybrid pines has always been a challenge (e.g. Mergen et al., 1965; Zobel, 1953). Little and Richter (1965) describe most of the identifiable traits of the hybrid pine as intermediate between the two parent species. Hicks (1973) determined that the most appropriate traits to identify hybrid pine were: needle length, fascicle sheath length, number of needles per fascicle, terminal bud width, cone length, and seed weight.

In 1989, Huneycutt and Askew used electrophoresis to show that the IDH (isocitrate dehydrogenase) enzyme was monomorphic and unique in shortleaf and loblolly pines, allowing easy and accurate identification of F1 hybrid pine. IDH electrophoresis was used by Edwards-Burke et al. (1997) to confirm 34 putative hybrid seedlings in Georgia stand; they further confirmed that all hybrid pines were sired by shortleaf pine pollen using a cpDNA restriction site marker.

In 1994, Xu took a wide survey of shortleaf pine genetic diversity from across the southern United States. Xu found, among other things, that the rate of hybridization in

shortleaf pines and loblolly pines was higher west of the Mississippi river (9.3% and 4.5%, respectively) than east of the Mississippi river (0% and 1.1%, respectively). Xu was also the first to determine hybridization using AFLP data in addition to the IDH enzyme. Edwards and Hamrick (1995) also found higher rates of hybridization west of the Mississippi river in shortleaf pine populations (4.6%) than east of the Mississippi river (1.1%). Raja et al. (1997) criticized the use of the IDH enzyme, since 16% of shortleaf pine sampled were polymorphic at this locus. They did not consider that 16% of the trees sampled may be hybrid.

Chen et al. (2004) used PCR-RFLP analysis of the *rbcL* gene to show that F1 hybrid pines were capable of backcrossing into their parent populations. They found 14% of their sampled trees to be hybrid pine. The authors also suggested that the previous estimates of hybridization (e.g. Edwards-Burke et al., 1997) may be lower than true rates in the wild, due to the more sensitive RFLP analysis. The authors conjectured that Raja et al.'s 1997 percent polymorphic rate (16%) may actually be the rate of hybridization in the trees Raja et al. sampled.

As methods became more sensitive, the projected rate of hybridization continued to be refined. Using AFLPs and IDH, Xu et al. (2008) identified a wide variance in hybridization rates in shortleaf pine populations, from 30% in one Arkansas seed source, to 0% in other sources. The average rate of hybridization in shortleaf pine west of the Mississippi river (16.3%) was higher than east of the Mississippi river (2.4%). Loblolly seed sources west of the Mississippi were higher (4.5%) than east of the Mississippi river (3.3%) using this method.

Stewart et al. (2010) developed the use of SSR markers (short sequence repeat markers) to identify shortleaf x loblolly pine hybrids. The authors asserted that SSR markers are more informative than AFLP or IDH based methods due to the larger amount of genetic information contained by SSR markers. Stewart et al. also found the same pattern of higher hybridization rates west of the Mississippi river than east. They explain this pattern by citing the more variable year-to-year fluctuations in climate, producing more frequent pollen-shed overlap between the two species. This is a phenomena which Hicks et al. (1972) reports having been observed without too much difficulty in Texas. Additionally, Schmidting (1971) found that western shortleaf pine sources shed pollen earlier than eastern shortleaf pine sources, providing yet another explanation for the increased hybridization west of the Mississippi River.

### **Alarming Increase in Hybridization**

Using SSR markers, Stewart et al. (2012) compared over 200 mature trees from the 1950s to over 300 seedlings and saplings (<10 years old) from the same counties using 11 loblolly pine populations and 10 shortleaf pine populations from around the southern United States. The older shortleaf pine and loblolly pine populations were made up of 3.3% and 4.5%, respectively. The younger cohort was composed of 46.7% hybrid pines in the shortleaf pine population and 27.3% hybrid pines in the loblolly population. Stewart et al. attributed this dramatic increase over a short time period to anthropogenic influences, such as habitat fragmentation and fire exclusion.

### **Shortleaf x Loblolly Hybrid Pine and Fire**

Lilly et al. (2012a) lent credence to Stewart et al.'s implication of fire exclusion as a potential cause of increased hybridization in the southern forest. They found that the

basal crook, which is hypothesized to aid shortleaf pine in resprouting following topkill by fire, is absent or weaker in the hybrid pine seedlings than in the pure shortleaf pine seedlings. Although the hybrid pine had similar resprouts as shortleaf pine after topclip, the resprouting potential is nullified if the dormant buds are destroyed by fire due to the lack of a protective basal crook. Will et al. (2013) also found that hybrid pine had higher resprouting rates than loblolly pine (although not as high as shortleaf) following topclip and propane torch girdling. Will et al. also found that the height to the lowest sprout was greatest in loblolly pine, followed by hybrid pine, and lowest in shortleaf pine. The authors concluded by suggesting that pure shortleaf pine likely have the morphological attributes to survive after fire - the hybrid pine's ability to resprout following fire was considered dubious.

Stewart et al. (2014) demonstrated that frequent fire does protect shortleaf pine from introgression by loblolly pine. The authors sampled seedlings growing under a mixed forest of shortleaf, hybrid, and loblolly pines with two different burning treatments. In the first treatment, fire was applied every other year for the last 3-4 decades; in the second treatment, fire had been excluded since the 1960s. In frequently burned stands, loblolly pine seedlings and saplings were absent from the seedling cohort, and hybrid pines were less common. The majority of seedlings in burned areas were shortleaf pine. In unburned stands, there was a greater mix of species, with shortleaf pine, hybrid pine, and loblolly pine all represented in the seedling and sapling cohort.

### **Shortleaf x Loblolly Hybrid Pine and Drought**

While much attention has been paid to the hybrid pine's pathogen resistance and fast growth, there is a conspicuous lack of information regarding its water use or tolerance for drought.

The only examination of the hybrid pine's short term water use behavior comes from Lilly et al. (2012a) who compared net photosynthesis ( $A_{\text{net}}$ ), stomatal conductance ( $g_s$ ), and intracellular  $\text{CO}_2$  ( $C_i$ ). While  $A_{\text{net}}$  and  $g_s$  did not vary between the three species,  $C_i$  (a measure of instantaneous water use efficiency) was significantly lower for shortleaf and hybrid pines than for loblolly pine (i.e. shortleaf and hybrid pine had higher instantaneous water use efficiency).

### **Implications of a Hybrid Forest**

With shortleaf pine stands in decline (South and Buckner, 2003), especially in the seedling class (Oswalt, 2011), and the rate of hybrid pines increasing in the seedling cohort (Stewart et al., 2012), there is potential for serious and possibly irrevocable damage to the shortleaf pine population. Elliott and Vose (2005) demonstrated that once shade tolerant, fire sensitive species become established under a shortleaf pine canopy, it can be difficult to regenerate shortleaf pine seedlings. This positive feedback cycle of fire sensitive plants creating conditions unfavorable for fire is called "mesophication" and once initiated, it can be very difficult to reverse (Nowacki and Abrams, 2008). Tauer et al. (2007) proposed that the combination of growing loblolly pine plantations, fire exclusion, and threat of introgression by loblolly pine via hybrid pines, put shortleaf pine at serious risk.

Shortleaf pine was once the dominant southern pine. The hybrid pine swarm that now imperils the genetic and phenotypic purity of the shortleaf pine population must be

better studied in order to understand and mitigate the threat that introgression poses to shortleaf pine forests. Specifically, testing the hybrid pine's tolerance to drought and fire disturbance will provide land managers with strategies to remove the hybrid pine from the seedling cohort and allow predictions on how an increasingly hybrid southern pine forest will react to these two frequent disturbances.

The 20th century was relatively benevolent to the southern forest. Precipitation increased from historic average levels (Cook et al., 2007; Frich et al., 2002; Karl and Knight, 1998) and mean fire return intervals increased as well (Fowler and Konopik, 2007; Nowacki and Abrams, 2008). We should seek to conserve the genetic and phenotypic advantages of hardy species like shortleaf pine that evolved in response to pressures like drought and fire. There is no reason to believe that the 21st century's climate will be as kind as the last century's climate. If our future brings more frequent drought (Romero-Lanko et al, 2014) or more frequent fire (Flannigan et al., 2000; Liu et al. 2010), we will need shortleaf pine present in our forests to buffer the deleterious effects of these disturbances.

This literature review sought to highlight the importance of both the shortleaf pine and loblolly pine species to the economy and ecology of the southern United States. In addition, it has highlighted the decline of the once dominant shortleaf pine forest and the rise of the more commercially productive loblolly pine forest. The shortleaf x loblolly hybrid pine is now threatening the remaining shortleaf pine population through competition and introgression. While some of the hybrid pine's traits have been studied, the hybrid pine's response to disturbances like fire and drought are not well characterized.



Lilly et al. (2012a) tested water use efficiency of the hybrid pine relative to the two parent species, but did not impose water stress on the seedlings. Similarly, Will et al. (2013) tested the resprouting ability of the hybrid by using a propane torch to girdle the seedlings, but this could not include the effect of the basal crook when estimating the hybrid pine's resprouting ability after natural topkill by fire. In this thesis, I aim to better understand the hybrid pine's responses to drought and fire - in doing so, we will have a better understanding of the factors that have allowed the hybrid pine to succeed in recent years, the implications of growing hybrid swarm, and how to manage these forests to restore the shortleaf pine forest.

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## CHAPTER III

### *Formatted for Trees - Structure and Function*

#### **The Basal Crook of Shortleaf Pine Seedlings is an Important Adaption to Resprout Following Topkill by Fire**

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#### **Abstract:**

Shortleaf pine (*Pinus echinata*) has the largest distribution of any southern pine in the United States but has seen considerable decrease in dominance in the last 100 years. Shortleaf pine is unique among the southern pines for its basal crook which is believed to confer fire tolerance by protecting the dormant buds that develop in the axils of primary needles. The basal crook protects shortleaf pine seedlings by lowering the dormant buds to near the ground level (Will et al, 2013), where surface fires are not as hot. However, shortleaf pine also has a higher resprouting capacity than other southern pines, including loblolly pine (*Pinus taeda*). This higher resprouting capacity of shortleaf pine confounds our interpretation of the basal crook's importance as an adaptation to resprout after fire.

We tested the basal crook's importance by exposing shortleaf pine's dormant buds to fire, which negated the crook, and comparing it to non-manipulated shortleaf pine. We also protected loblolly pine's dormant buds from fire by mounding mineral soil around the stem, which simulated the function of the basal crook, and compared that response to non-manipulated loblolly pine. We also topclipped seedlings of each species to test resprouting capacity in the absence of fire. Exposed basal crook shortleaf and non-manipulated loblolly pine failed to resprout following fire, i.e., seedlings with exposed dormant buds failed to resprout. Non-manipulated shortleaf pine and simulated basal crook loblolly pine resprouted after topkill by fire, i.e., seedlings with protected dormant buds successfully resprouted. Both species resprouted after topclipping. These results confirm that the basal crook is an indispensable morphological feature which confers a large proportion of shortleaf pine's fire tolerance. Without the basal crook, shortleaf pine seedlings would likely be as vulnerable to fire as its relative, loblolly pine.

**Keywords:** loblolly pine, shortleaf pine, fire tolerance, basal crook, resprouting

**Key Message:** Shortleaf pine's ability to resprout after fire is facilitated by its basal crook which protects dormant buds from fire.

## Introduction

Shortleaf pine (*Pinus echinata* Mill.) is a fire-adapted species of great ecological importance in the southern forests of the United States. Shortleaf pine's historic range stretches north from New Jersey south to Florida, and west to Texas and Oklahoma, giving it the widest range of any southern pine in the United States. While once broadly distributed, the abundance of shortleaf pine has been greatly reduced; Oswalt (2011) quantified the decline as a 52% loss of shortleaf pine forests between the 1980s and 2010. Furthermore, the majority of remaining shortleaf pine forests are mature, i.e. regeneration (natural or otherwise) is not occurring. Factors for this decline include land use change, introgression through hybridization, the interruption of historic fire patterns, and replacement with more productive timber species (Dipesh et al., 2015; Lawson, 1990; South and Buckner, 2003; Tauer et al., 2007; Williston, 1972).

While shortleaf pine does not grow as quickly as some associates, it has several adaptations which allow it to vigorously respond after disturbance. Shortleaf pine's most important feature may be the basal crook, first described by Mattoon (1915). The basal crook is a portion of stem just above the root collar that forms when the stem bends by forming compression and tension wood near the hypocotyl region so that it is flat against the ground before growing up vertically again just beyond the hypocotyl region. The seedling usually develops the basal crook in the first year of its life and typically loses the crooking feature as the stem grows to a diameter large enough to withstand a surface fire. The basal crook presses dormant buds, which are set in the axils of the primary needles, against the ground. Duff and litter may accumulate around the crook, at times burying the basal crook. In addition, buds on the underside of the crook are pressed against the soil.

When a shortleaf pine is topkilled by fire, these dormant buds are presumably protected from the heat of the fire (Lilly et al., 2012b). The surviving buds sprout vigorously and add height at a faster rate than unburned seedlings. After burning, resprouted shortleaf pine seedlings can respond quickly enough to outgrow competitors, including unburned hardwoods (Cain and Shelton, 2000).

The basal crook has been thought to impart fire tolerance to shortleaf pine seedlings. However, other factors influence the ability of shortleaf pine seedlings to recover following topkill. For example, shortleaf pine resprouts more quickly after topclipping than the closely related loblolly pine (*Pinus taeda* L.) (Liu et al., 2011). Shortleaf pine also produces more sprouts than loblolly pine when topclipped (Lilly et al., 2012b) and maintains the ability to successfully resprout to an older age than loblolly pine (Campbell and Station, 1985). The linked attributes of the basal crook and greater sprouting capacity in shortleaf pine makes it difficult to determine the functional importance of the basal crook and the role that the crook plays in survival after topkill from fire.

With increased risk of drought associated with climate change in the southeastern United States and the enhanced risk that wildfire poses to forests (Flannigan et al., 2000), determining the relative importance of characteristics that confer fire tolerance to shortleaf pine is important. In particular, understanding the role of the crook is important to develop management strategies to reduce the prevalence of shortleaf x loblolly pine hybrids. There has been a recent surge in the hybrid component across the southern forest (Stewart et al., 2012). The hybrids have a weak crook that does not lower dormant buds to the soil surface but hybrid pines have resprouting potential resembling shortleaf pine

(Lilly et al., 2012a). Determining the function of the basal crook will give valuable insights regarding the vulnerability to fire of the hybrid seedlings and saplings.

The objective of this study was to determine the importance of the basal crook by manipulating shortleaf pine seedlings to expose the dormant buds to fire in a manner similar as if they did not have a crook. As a comparison, we protected dormant buds of loblolly pine by covering with mineral soil to simulate the effect of a crook and measured the sprouting response to fire.

## Methods

Shortleaf pine and loblolly pine seeds were produced at the Kiamichi Forestry Research Station in Idabel, Oklahoma, USA (33.8942, -94.7519). Six open pollinated shortleaf pine trees and six open pollinated loblolly pine trees were used to produce seeds. Seeds were collected from cones and stored in a -20 °C freezer.

On 24 January 2013, seeds were removed from the freezer and soaked in water for 12 hours at approximately 4 °C. Seeds that sank were selected for stratification. Seeds were stratified in a refrigerator in a moist paper towel in a plastic bag at 4 °C for 56 days. Seeds were planted at the Kiamichi Forestry Research Station, Idabel, Oklahoma on 28 March 2013. The field was approximately 0.2 ha, and had been tilled prior to planting. The soil is 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 percent. In total, 72 spots were planted across the field. At each planting spot, 10-15 seeds were covered by 1 to 2 cm of mineral soil. Spots were planted with 2 m spacing along a north/south and east/west axis. Each spot was marked by numbered, metal tags attached to a stake. Chicken-wire enclosures were placed over each planting site and staked into the ground to prevent herbivory.

Throughout the summer of 2013, the field was watered and competition was controlled. 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 spots 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 18 March 2014, seedlings were treated either with top clipping or by burning.

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 placed 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.

Loblolly pine also had three treatments applied: Burned, Protected, and Clipped. 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 as loblolly pine lacks a basal crook. Protected loblolly pine had mineral soil mounded up against the stem, covering the dormant buds with a layer of soil 2 to 4 cm above the height of the dormant buds. The soil was removed within an hour after fire. The Protected treatment simulated the basal crook, which protects buds from fire damage by pressing them against or under the soil.

Burning the seedlings consisted of piling approximately 1 kg of air dried pine needle litter in a 0.5 m<sup>2</sup> area around the seedling. Woody material and cones were removed from the litter when placing the litter under the seedlings.



Of the 72 planting spots, 31 had healthy, live seedlings. Loblolly pine had 5 sites (9 seedlings) that were Burned, 6 sites (13 seedlings) that were Protected, and 6 sites (11 seedlings) that were Clipped. Shortleaf pine had 4 sites (7 seedlings) that were Burned, 6 sites (10 seedlings) that were Exposed, and 4 sites (4 seedlings) that were Clipped. On 24 April 2014 seedlings were examined for resprouting. For each seedling, both the resprouting status and the number of resprouts were recorded.

Before burning, thermocouples (type K) were placed at ground level next to the seedling stem. Pine straw was laid over the wire. The thermocouple wires were attached to a data logger with temperature recorded every 0.5 seconds during fire.

## Results

At time of burn, loblolly pine ( $25.8 \text{ cm} \pm 2.2 \text{ SE}$ ) were taller than shortleaf pine ( $19.5 \pm 3.0 \text{ SE}$ ) ( $p=0.09$ ). Likewise, ground line diameter (GLD) of loblolly pine ( $8.4 \pm 0.6 \text{ SE}$ ) was greater than shortleaf pine ( $6.4 \pm 0.8 \text{ SE}$ ) ( $p=0.06$ ).

Fires were backed into seedlings and lasted approximately six minutes from ignition to extinction. There were no differences in fire intensity between treatments ( $p=0.14$ ). Maximum fire temperatures near ground level averaged  $339 (\text{SE} \pm 28) ^\circ\text{C}$ .

Of the Burned loblolly and shortleaf pines, 71% of shortleaf pine resprouted after treatment and no loblolly pine seedlings survived (Table 3.1). Exposed shortleaf pine did not resprout when burned with an exposed basal crook. Forty-six percent of Protected loblolly pine survived the fire and resprouted. In the Clipped treatment, 100% of loblolly and shortleaf pines resprouted after coppice. Of seedlings that resprouted, shortleaf pine ( $6.78$  resprouts per surviving seedling) had more sprouts than did loblolly pine ( $4.17$ ) ( $p=0.0001$ ). Clipped shortleaf pine had a greater number of resprouts ( $7.75$ ) than Clipped loblolly pine ( $3.90$ ) ( $p=0.01$ ).

## Discussion

We separated the crook effect from sprouting capacity by manipulating shortleaf and loblolly pine seedlings. Our results indicate that the basal crook is important for shortleaf pine's resprouting ability after fire. The basal crook lowers the height of the dormant buds and protects them by insulating the buds against the ground, reducing damage from heat. This morphological feature is a critical component in shortleaf pine's tolerance to fire and without it, shortleaf pine's response to fire would be similar to loblolly pine's response, i.e., no shortleaf pine with exposed crooks survived.

In the Protected treatment, only about half the loblolly pine had live sprouts when examined 45 days after burning even though the moist mineral soil surrounding the dormant buds was sufficient to insulate them from the lethal heat of the fire. Compared to the Clip treatment (in which all seedlings survived) 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 water stress as foliage above the girdle may still lose water. In the current study, some loblolly pine seedlings in the Protected treatment were not completely incinerated by the fire, leaving scorched needles above the charred branches and stem which would be similar to the girdling treatment in Will et al. (2013). If these non-burned portions continued to lose water, the seedlings may have become too desiccated to successfully resprout.

Burned shortleaf pine responded as expected; the majority of seedlings were able to resprout vigorously after topkill by fire. Burned loblolly pine did not survive, despite

having larger GLD and heights than the resprouting shortleaf pine. In Clip treatments, both one-year-old loblolly and shortleaf pines resprouted, with all seedlings surviving, which is similar to previous studies (Lilly et al., 2012a; Little and Somes, 1956).

Shortleaf pine did produce more resprouts than loblolly pine after topkill, as previously reported (Lilly et al., 2012a; Will et al. 2013).

Compared to the Clip treatment (100% survival), not all shortleaf pine seedlings survived burning. While shortleaf often resprout following fire, some do not probably because they are exposed to lethal heat even though the crook holds dormant buds lower. Lilly et al. (2012b) found that 40% of burned shortleaf pine seedlings in a prescribed fire in the Ouachita National Forest did not resprout and that mortality was related to both higher fire intensity and seedling size (Lilly et al., 2012b). Damage from fire could include scorching the dormant buds if the wind, fuel load, or soil topography allowed for the buds to be exposed to damaging heat. Another explanation could be that burned seedlings with scorched needles possibly experience desiccation after burning, relative to top clipping, as described above.

Prescribed fire can shift seedling populations from a mix of loblolly, shortleaf, and shortleaf x loblolly pine to almost pure shortleaf pine (Stewart et al., 2014). Results of the current study suggest that shortleaf x loblolly pine hybrids probably do not survive fire because they lack a strong crook that keeps the buds at the soil surface even though the hybrids have sprouting capacity similar to shortleaf pine (Lilly et al., 2012a; Will et al., 2013). Shortleaf pine's basal crook, and the fire tolerance that the basal crook confers, makes it a crucial component in regards to resiliency. The forests of the southeastern US will be exposed to a multitude of stresses in the 21st century, including fire (Flannigan et

al., 2000; Liu et al., 2010; Nowacki and Abrams, 2008). Southern forests with a pure shortleaf pine population will persist in the face of fire, while forests with significant loblolly pine components are at increased risk of fire.

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**Table 3.1** Resprouting success of one-year-old shortleaf and loblolly pine seedlings following topkill. Burned consisted of burning unaltered seedlings. Exposed consisted of burning shortleaf pine whose basal crooks were exposed. Protected consisted of burning loblolly pine seedlings whose dormant buds were protected by mounding mineral soil. Clipped consisted of cutting the top off above dormant buds.

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



## CHAPTER IV

*Formatted for Trees - Structure and Function*

### **Are Shortleaf x Loblolly Hybrid Pine Seedlings as Drought Tolerant as Shortleaf Pine Seedlings?: Allometry and Transpiration Responses to Water Stress.**

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#### **Abstract:**

Shortleaf pine (*Pinus echinata* Mill.) and loblolly pine (*Pinus taeda* L.) are both species of great importance in the southeastern forests of the United States. Hybridization between shortleaf and loblolly pines has dramatically increased in the last 60 years which threatens the genetic integrity of the shortleaf pine. Shortleaf pine is more drought tolerant than loblolly pine but the drought hardiness of the hybrid pine is not known. If the hybrids have similar drought tolerance, they may have the ability to displace shortleaf pine on more xeric sites. To determine the drought tolerance of the shortleaf x loblolly pine hybrids, we measured the transpiration of the hybrid pine seedlings to soil drying and compared it with both loblolly and shortleaf pine seedlings. We also determined

seedling allometry in response to drought for shortleaf, hybrid, and loblolly pine seedlings.

We found no differences in the soil moisture at which seedlings of different species began to limit transpiration. Drought treatment seedlings had a greater proportion of foliage than well watered seedlings. Shortleaf pine seedlings had a greater proportion of biomass allocated to coarse roots and hybrid pine was intermediate between the parent species. Our results suggest that the transpiration response of shortleaf pine and hybrid pine is similar to loblolly pine when exposed to water stress. However, a greater proportion of coarse root may allow shortleaf or hybrid pines to wait out drought by relying on a greater surplus of below ground carbohydrate.

**Keywords:** shortleaf pine, loblolly pine, hybrid, water stress, allometry, partitioning, drought

**Key Message:** shortleaf x loblolly hybrid pine seedlings may be more drought tolerant than loblolly pine seedlings due to greater below ground partitioning, similar to shortleaf pine seedlings

**Introduction:**

Shortleaf pine (*Pinus echinata* Mill.) and loblolly pine (*Pinus taeda* L.) are genetically similar, sharing a common ancestor just one or two divergences back along the *Pinus* evolutionary tree (Gernandt et al., 2005). Despite their genetic similarity, loblolly pine is less drought tolerant than shortleaf pine (Sayer et al., 2005; Schopmeyer, 1939). Perhaps related to its greater drought tolerance, shortleaf pine is slower growing than the loblolly pine. Slow growth rates are an ecological adaptation to unfavorable conditions such as drought (Parsons, 1968). In Scots pine (*Pinus sylvestris* L.) genotypes with slow growth rates also had higher drought tolerance characteristics: smaller size, more biomass allocated to roots, and higher water use efficiency (Cregg and Zhang, 2001). The slower growth of the shortleaf pine seedling puts it at a competitive disadvantage on sites where loblolly pine can successfully establish (Kramer, 1943). However, the slower growth rate may be an adaptation that allows shortleaf pine to persist on sites with low nutrient and water availability (Lawson, 1983).

Given their genetic similarity, hybridization has likely always occurred between the two species (Tauer et al., 2007). Shortleaf x loblolly hybrid pines are typically intermediate in morphological traits and may have a resistance to pathogens which are deleterious to the parent species (Cotton et al., 1975). More recently, artificial F1 and F2 hybrids have been created in an effort to breed tolerance to fusiform rust (Kraus, 1986; La Farge and Kraus, 1980). While morphometric methods were traditionally used to identify putative hybrids, new methods are currently being employed to identify hybrid pines (Edwards-Burke et al., 1997). Using microsatellites, Stewart et al. (2012) found that hybrid seedling numbers have dramatically increased in the last 60 years. Stewart et al.

compared the genetic makeup of mature trees that were seedlings in the 1950s to current seedling cohorts. While the 1950 shortleaf pine and loblolly pine forests were only 3% and 4% hybrid, respectively, the present seedling cohort is 46% and 23% hybrids.

Loblolly and shortleaf pines are both ecologically and economically important in the southern forests of the United States (Schowalter et al., 1981). Loblolly pine is the most commercially widespread pine in the southern United States, with 1.2 billion seedlings planted annually (McKeand and Allen, 2005). Together, the southern pines cover over 71 million acres of forest in the United States. These early successional pine forests provide habitat for wildlife; additionally, their fine litter encourages frequent fire - a disturbance which is vital to sustaining the biodiversity of the forests in the southern United States. Allowing pine forests to transition to a hardwood dominated forests can have negative impacts on plant biodiversity and wildlife (Blair and Feduccia, 1977).

Hybridization between shortleaf and loblolly pines may lead to less resilient pine forests, as introgression between the two species might dilute specific phenotypic traits. A less resilient pine forest is one whose continued persistence in the drought and fire prone southeastern United States is endangered. Climate change projections are for a warmer and drier future across the southern pine range (Romero-Lankao et al., 2014). How will an increasingly hybrid forest respond? If hybrid pines are as drought tolerant as their shortleaf parents, it would be reasonable to expect that the hybrid pine would gain an even larger presence in the southern forest and that the southern pine component will persist. However, if the hybrid pine is not as drought tolerant as their shortleaf pine parents, drought might eliminate the southern pine component where hybrid pines established during periods of higher moisture availability. Additionally, biotic stresses

(e.g. pine beetle) may be more likely to severely impact forests that are already suffering from water stress (Weed et al., 2013). A less drought tolerant pine component of the southern forest exposes the southern forest to the risk of large mortality events.

We sought to determine the drought tolerance of the shortleaf x loblolly hybrid pine seedlings and compare it directly to both loblolly and shortleaf pines. The increased presence of hybrid pine in what was historically shortleaf pine forest suggests that hybrid pine may have similar water stress tolerance as shortleaf pine. There is some evidence that hybrid pine has a leaf-level water use efficiency (carbon gain per water loss) that is closer to shortleaf pine than loblolly pine (Lilly et al., 2012). However, the seedlings in that study had not been subjected to any water stress and there was no difference in xylem water potentials among loblolly, shortleaf, or hybrid pines. To quantify water stress response, we measured the changes in seedling allometry to prolonged water stress. We hypothesized that foliage and stem would be reduced in drought stressed seedlings and fine root biomass would increase in drought stressed seedlings. Furthermore, we hypothesized that shortleaf pine seedlings would have a relatively greater component of fine root while loblolly pine would have a relatively greater component of stem, with the hybrid pine intermediate. We predicted that drought stressed shortleaf pine would have a larger change in allometry in response to drought than loblolly pine, with hybrid pine intermediate. In addition, we measured the Normalized Transpiration Rate (NTR) (Jyostna Devi et al., 2009) in response to reduced soil water availability. We hypothesized that shortleaf pine would respond to drying soil by reducing its NTR earlier than loblolly pine, with hybrid pine's response intermediate.

**Methods:**

Shortleaf pine, loblolly pine, and shortleaf x loblolly pine seeds were produced at the Oklahoma Forestry Services seed orchard in Idabel, Oklahoma, USA (33.8942, -94.7519). Both shortleaf pine and loblolly pine seeds were collected from open pollinated cones from six shortleaf and six loblolly pines. Hybrid pine seeds were created through controlled pollination of six loblolly by five shortleaf parent trees. Eight hybrid families were produced from the crosses. Shortleaf pine pollen was used to fertilize loblolly pine. Seeds were collected from cones and stored in a -20 °C freezer.

On 24 January 2013, seeds were removed from the freezer and allowed to soak in water for 12 hours at approximately 4 °C. After 12 hours, seeds that sank were selected for stratification. Seeds were then stratified in a refrigerator wrapped in a moist paper towel and placed in a plastic bag, at 4 °C for 75 days.

Soil planting medium was prepared by mixing 2 parts of soil from Idabel, OK with 2 parts coarse sand (Coarse Sand, Quikrete, OH, USA), and 1 part peat moss (Majestic Earth, Sun Gro, MA, USA). Soil series of the native soil used were a mixture of Adaton loam (Fine-silty, mixed, active, thermic Typic Endoaqualfs) and Kullit fine sandy loam (Fine-loamy, siliceous, semiactive, thermic Aquic Paleudults). Before mixing, soil was screened through a 1 cm<sup>2</sup> mesh to remove large clumps, roots, or other debris. Components were mixed in a 100 gallon concrete mixer and poured into 80 approximately 5.7 liter pots (22 cm height x 22 cm diameter). Pots were watered beyond field capacity before planting.

In total, there were eight hybrid pine full-siblings families, six loblolly pine half-sibling families, and six shortleaf pine half-sibling families. Each family was planted in

four pots. Each pot was planted with 4-5 seeds on 10 April 2013 in a greenhouse in Stillwater, OK (36.119379, -97.104179). In total, 80 pots were planted - each pot had a matching partner, yielding 40 pairs. Partner pots were planted with the same seed source and were placed next to one another on greenhouse benches along a north-south axis. In the greenhouse, daytime temperature was set to 24 °C and nighttime temperature was set at 20 °C. Pots were watered daily with a fine mist until seedlings had established, at which point they were watered on an every-other day regimen until the beginning of the experiment. Seedlings were treated every 14 to 21 days with a (24-8-16) fertilizer with micronutrients (B, Fe, Cu, Mn, Mo, Zn) (Scotts Miracle Grow Products Inc, OH, USA). Fertilizer was applied at a concentration of 0.66 mL per liter.

Seedlings were allowed to grow until July 2013, at which point the hardiest seedling was selected and the others were either gently removed from the soil or clipped. Seedlings were selected based on their height, the number of needles, and the coloration of their foliage. After thinning, steel welding rods were installed to measure TDR (time-domain reflectometry). Rods were 15 cm in length, had a diameter of 1.59 mm, and were inserted 3 cm apart from one another near the base of the remaining seedling. In each pair of pots, one pot was randomly selected for drought treatment. After the seedling thinning, but before the treatments began, two seedlings died. This left 78 total seedlings in the study and 38 pairs. All of the remaining seedlings survived until the end of the study. Just before the beginning of the drought period, we measured height (to the nearest mm) and ground line diameter (GLD; to the nearest 0.1 mm).

The seedlings were exposed to three episodes of drought: the first from 4 September 2013 to 15 October, the second from 18 October 2013 to 15 November 2013,

and the third from 17 November 2013 to 8 January 2014. Between each drought event, all pots were watered to field capacity and given fertilizer. No fertilizer was added during the dry down events. During the third dry down event, gravimetric transpiration measurements were collected. After the third dry down event, seedlings were destructively harvested. In total, seedlings were exposed to 122 days of drought treatment.

During the first dry down, drought treatment pots were allowed to reach 2% soil moisture (volumetric water content measured using TDR). Time domain reflectometry was measured with a cable tester (Tektronix TDR Metallic Cable Tester, Model 1502B). In addition to measurements of TDR, pots were weighed on a scale (to the nearest gram). When TDR measured less than 2% soil Volumetric Water Content (VWC), drought pots were given 300 mL of water which returned them to approximately 14% VWC. After every addition of 300 mL of water, pots were not watered again until they once again reached 2% VWC. During this time, control pots were watered on a two or three day regime to bring them back to their initial weight. The soil moisture of the control pots were measured by TDR and ranged from 25-35%.

Both drought and well-watered pots were fully watered on 16 October 2013. After allowing the pots to drain excess water, pots were fertilized as described above. Pots were watered again to full capacity and allowed to drain. Two days later, on 18 October all pots were weighed. The weight of each pot was recorded as the starting weight, and a plastic bag was put around each pot to prevent evaporation of water from the soil surface. Change in pot weight could only be explained by water loss via transpiration.



The second dry down began on 18 October 2013. All pots were weighed in a two hour period before sunrise at approximately 06:00 CST. Well watered pots were returned to a weight 100 grams less than their starting weight, measured to the nearest gram. TDR was not measured, but water was added to the drought treatment pots when the normalized transpiration rate reached 0.10 (see below). After reaching NTR of 0.10, 250 mL of water was added. After that point, water was added based on gravimetric water loss for the remainder of the dry down period. Gravimetric water loss had been correlated with volumetric water content for each seedling so that volumetric water content could be inferred by pot weight (i.e. when a seedling reached a certain known weight, it was at or below 2% volumetric water content). During the second down period, average daily temperature in the greenhouse was 16.0 °C with an hourly standard deviation of 3.0 °C. Maximum and minimum temperatures were 26.5 °C and 8.2 °C, respectively. The last pots reached an NTR of 0.10 on 15 November 2013. All pots were watered to field capacity and allowed to drain. Pots were then fertilized and again watered to field capacity. Pots were allowed to drain excess water for two days.

The third dry down period began on 17 November 2013. Pots were initially weighed and again bagged to prevent evaporation. All pots were weighed on a 48 hour schedule after sunset around 19:00 CST. Average temperature in the greenhouse was 10 °C with a standard deviation of 3.4 °C. Maximum and minimum temperatures were 23.5 and -0.5 °C, respectively.

The daily change in weight of drought pots was compared to well watered companion pots (same family located adjacent to drought pot) to calculate a ratio for the pair called the Normalized Transpiration Ratio (NTR), which was expressed as a fraction

(Jyostna Devi et al., 2009). This fraction started at approximately 1.0 and became smaller as drought pots began to experience drought stress and use less water.

Initially, partner trees did not use equal amounts of water as they varied in size, leaf area, and transpiration rate. In order to begin the dry down with a water use fraction of 1.0, the first three days' water use for each pair were averaged. The daily fraction was divided by this first three day fraction to calculate the NTR.

$$\text{Normalized Transpiration Rate (NTR)} = \frac{\text{daily drought use/daily well watered use}}{\text{first three day average of (drought/well watered)}}$$

After the initial first three days of the dry down period, well watered pots were returned to their initial weights minus 100g every other day. Drought pots were given no water until their Normalized Transpiration Rate reached 0.10. When a drought pot reached 0.10 NTR, the soil was considered to have no transpirable soil moisture left. At this NTR, pots were given 300 mL of water, and then allowed to dry down again until approximately 2% soil moisture (by weight of pot, using weight that corresponded to 2% VWC) when 300 ml was again added once again. This was repeated until all other drought pots reached 0.10 NTR.

Using Jyostna Devi et al. (2009)'s fraction of transpirable soil water (FTSW) equation, NTR was plotted against daily changes in transpirable soil moisture. At NTR of 0.10, FTSW is considered to equal zero.

$$\text{Fraction of Transpirable Soil Water (FTSW)} = \frac{(\text{daily weight} - \text{final weight})}{(\text{initial weight} - \text{final weight})}$$

The points were fit into a segmented regression using SAS 9.3. Two line segments were regressed per pair of pots. During the early part of the dry down, the line segment was fairly horizontal, as the NTR was fairly constant at high soil water content. During the later stages of the dry down, the line sloped downward; the drought pots were reducing their water use compared to the well watered pots. The point where the horizontal and diagonal line crossed is the join.

The FTSW corresponding to the join was used as the experimental unit in a one-way ANOVA with species as the main effects (n=16 for hybrid pine, n=12 for loblolly pine, n=10 for shortleaf pine). The last pot reached an NTR of 0.10 on 8 January 2014.

Beginning on 21 January 2014 each seedling was harvested and separated into component parts: needles, branches, stem, coarse roots, and fine roots. Fine roots were classified as any root with a diameter less than or equal to 2.0 mm. Needles were harvested by pulling the entire fascicle from the branch or stem. Roots were cleaned of clinging clay particles and organic matter. Loose soil was screened for broken root segments. Seedling stems were cut parallel to, and just above, the soil surface. Seedling pairs were harvested within the same hour. After each day of harvest, the components were placed into a paper bag and put in a drying oven at approximately 60 °C. Paper bags were held in drying ovens until they were processed, approximately 10 days later. The last seedlings were harvested on 31 January 2014.

After all seedlings had been harvested, the paper bags containing the components were placed into an oven between 60-65 °C until constant weight and their contents then weighed to the nearest 0.01 g. Due to inherent differences in resource allocation as seedlings mature, biomass partitioning in larger trees is different than for a smaller

seedling (e.g. a larger tree may devote a larger percent of carbon to stem growth than a smaller tree). To correct for size differences in biomass partitioning, ANCOVA (Proc Mixed, SAS 9.3) with the main effects of species and water treatment was used to compare the component values at the same total biomass. This allowed for direct comparison among species at the same size. The data were log transformed to normalize the residuals. After the log transformation, the components' LS means were directly compared if there was no difference in the slope of the relationship between the component biomass and total biomass (n=16 for hybrid, n=12 for loblolly pine, n=10 for shortleaf pine).

## Results

Both species ( $p < 0.04$ ) and water treatment ( $p < 0.001$ ) significantly affected ground line diameter (GLD) growth during the course of the drought treatments. The hybrid pine seedlings and shortleaf pine seedlings grew less in GLD (both 5.3 mm) than did loblolly pine (6.1 mm). Drought treated seedlings grew significantly less GLD (4.3 mm) than their well watered siblings (6.8 mm). There was an interaction between treatment effects and species effects on height growth during the course of the drought treatments ( $p = 0.01$ ). Well watered loblolly pine height growth (39 mm) was less than well watered hybrid pine (79 mm) and well watered shortleaf pine (95 mm). Drought treated loblolly pine (33 mm) experienced similar height gains as drought treated hybrid pine (21 mm) and drought treated shortleaf pine (28 mm).

At time of harvest, ground line diameter (GLD) varied between species ( $p = 0.02$ ) and treatments ( $p < 0.0001$ ). Loblolly pine seedlings (12.5 mm) had significantly greater final GLD than shortleaf pine (10.6 mm) while hybrid pines (11.5 mm) were similar to both parent species (Figure 4.1). The drought treatment had a smaller final GLD (10.1 mm) than the well watered treatment (12.9 mm) (Figure 4.1). For final height, the effects of species and water treatment interacted ( $p = 0.02$ ). This interaction occurred because the height of shortleaf pine seedling and hybrid pine were negatively affected by the drought treatment, but loblolly pine seedlings were not (Figure 4.2). Drought treatment shortleaf pine seedlings were 36% shorter than their well watered siblings ( $p < 0.0001$ ); hybrid pine seedling height was stunted by drought by 13% ( $p = 0.05$ ). Loblolly pine seedling height was not significantly diminished by drought (5% reduction) ( $p = 0.46$ ).

With the exception of coarse root biomass and branch biomass, loblolly pine and hybrid pine had more biomass at the end of the experiment than shortleaf pine. Species differed in total biomass ( $p=0.02$ ) with loblolly (51.7 g) and hybrid pines (51.6 g) both larger than the shortleaf pine (40.3 g) (Table 4.1). Likewise, fine root biomass was greater in loblolly (16.0 g) and hybrid (14.9 g) pines compared to shortleaf (12.1 g) pine ( $p=0.03$ ). Stem biomass was greater in loblolly (8.5 g) and hybrid (6.9 g) pines than in shortleaf (4.5 g) pine ( $p=0.008$ ). Similar to patterns in stem biomass, foliage biomass was greater in loblolly (17.8 g) and hybrid (18.8 g) pines than in shortleaf (12.6 g) pine ( $p=0.03$ ). In contrast, shortleaf pine (9.4 g) had greater coarse root than loblolly pine (7.3 g) ( $p=0.05$ ) while hybrid pine (8.5 g) had intermediate values that were statistically similar to both parent species. Drought stress reduced total, fine root, coarse root, stem, and foliage biomass compared to the well watered treatment ( $p<0.0001$ ). Branch biomass did not differ among species ( $p=0.08$ ), however species and treatment did exhibit a significant interaction ( $p = 0.009$ ) because drought reduced branch biomass for hybrid ( $p<0.0001$ ) and shortleaf pine ( $p<0.0001$ ), but not loblolly pine ( $p=0.19$ ).

The amount of total biomass partitioned to fine root was consistent among species and treatment, i.e., the slope of relationship between  $\log(\text{total biomass})$  and  $\log(\text{fine root})$  did not statistically differ among species or treatments (Table 4.2, Figure 4.3). When the amount of fine root per total biomass was compared at the mean total biomass (LSMean), differences for fine root biomass were not statistically different between species or treatments (Table 4.3, Table 4.4) indicating that partitioning to fine root was similar among seedlings in the study.

For coarse root biomass, the slope of the relationship between  $\log(\text{total biomass})$  and  $\log(\text{coarse root})$  did not statistically differ among species or treatments (Table 4.2, Figure 4.4A and 4.4B). Both treatment and species influenced coarse root biomass partitioning as indicated by differences in LSMeans (Table 4.3, Table 4.4). Shortleaf pine had significantly more coarse root biomass than hybrid pine (13% greater for shortleaf) and loblolly pine (21% greater for shortleaf); hybrid pine had significantly more coarse root than loblolly pine (8% greater for hybrid). Partitioning to coarse root was greater for the watered treatment seedlings than for the drought treatment seedlings (12% greater).

For stem biomass, the slope of  $\log(\text{total biomass})$  and  $\log(\text{stem})$  relationship differed significantly among species (Table 4.2). At low total biomass, loblolly pine seedlings had greater stem biomass. However, this partitioning difference decreased when comparing the larger seedlings across the three species (Table 4.5, Figure 4.5). The slope of the relationship for shortleaf pine seedlings (1.36) was statistically similar to hybrid pine (1.45). The slope for shortleaf and hybrid pines were both greater than for loblolly pine seedlings (1.11) (Table 4.5). The slopes for the different watering treatments did not statistically differ when comparing the relationship between  $\log(\text{total})$  biomass and  $\log(\text{stem})$ .

The slope of the relationship between  $\log(\text{total biomass})$  and  $\log(\text{branch})$  did not differ among species or treatments (Table 4.2, Figure 4.6). There was a significant interaction between species and treatment when comparing LSMeans (Table 4.3). The interaction occurred because loblolly pine had increased branch biomass under drought (18% increase) compared to the well watered seedlings ( $p=0.01$ ) while shortleaf ( $p=0.05$ )

and hybrid pine ( $p=0.051$ ) seedlings had declines in branch biomass of 5% and 10% respectively (Table 4.4).

The slope of  $\log(\text{total biomass})$  and  $\log(\text{foliage})$  did not differ between species or treatments for the foliage component (Table 4.2, Figure 4.7). Drought treatment significantly increased partitioning to foliage by 5% (Table 4.3, Table 4.4). Partitioning among species did not differ.

During the drying period, the Normalized Transpiration Rate (NTR) was initially stable as the Fraction of Transpirable Soil Water (FTSW) declined. When the seedlings began to limit transpiration in response to soil drying, the NTR declined linearly. Figure 3.8 portrays a representative relationship between NTR and FTSW. Loblolly pine seedlings began to limit transpiration at a FTSW of 0.73 ( $SE=0.02$ ), hybrid pine at FTSW of 0.70 ( $SE=0.02$ ), and loblolly pine at FTSW of 0.77 ( $SE=0.01$ ); these differences were not significantly different from one another, indicating no differences between seedling transpiration patterns in response to decreasing soil moisture ( $p=0.15$ ).



## Discussion

The reduction in growth due to water stress we measured is consistent with previous research in pine seedlings (e.g. Teskey et al., 1987; Torreano and Morris, 1998). Drought reduces growth due to reduced turgor and reduced photosynthesis. Reduced photosynthesis in response to drought, due to stomatal and nonstomatal factors, was likely a mechanism related to reduced biomass in this study because the relative difference between drought stressed and well watered seedlings were greatest in the plant component that stores the greatest amount of starch, i.e., coarse root (48% reduction in biomass) (Gholz and Cropper Jr, 1991) which probably indicates less carbon available for storage. Components which store a smaller fraction of carbohydrate, e.g. stem (46% reduction in biomass), fine root (34% reduction in biomass) and foliage (24% reduction in biomass), experienced smaller relative decreases in biomass due to drought.

The drought treatments did not start until early September, after most of the seedling height growth and foliage development had been completed. For instance, the drought and well watered seedlings gained only 8.5% and 16.5% of their final height during the experimental period, respectively. Even though most height development had been completed before the treatments began, drought still reduced height growth in shortleaf and hybrid pines. The lack of a decline in height growth of loblolly pine (and smaller height growth of well watered loblolly pine during the period) could be due to differences in the phenology of flushing. When examining GLD effects, drought and well watered seedlings gained 42% and 52% of their final GLD during the experimental period, indicating continued growth of stem diameter throughout the experimental period.

Greater size of loblolly pine than shortleaf pine, with the hybrids intermediate, was expected based on previous research. Loblolly pine grows larger than shortleaf pine, especially at a young age (Williston, 1972). Even when loblolly pine was planted beyond its range, it attained greater heights and volumes than the native shortleaf pine (Dipesh et al., 2015). Previous studies of shortleaf x loblolly pine hybrids using the same genotypes as the current study found intermediate growth rates of the hybrids compared to the parent species (Lilly et al., 2012; Will et al., 2013).

Inherent size differences among species and treatments in our study confounded direct testing of partitioning to different biomass components because partitioning changes with seedling size (Bongarten and Teskey, 1987; Ledig et al., 1970). By using log transformation and ANCOVA to account for differences in size, we were able to test for differences in biomass partitioning for seedlings of the same total biomass.

The similarity in fine root partitioning among species and treatments was unexpected. Because of the role of fine roots in water uptake and the starch storage, we had hypothesized that shortleaf pine, which is more drought tolerant than loblolly pine (Lawson, 1990), would have proportionately more fine root biomass. Typically, fine roots develop to absorb water and nutrients in dry or nutrient poor conditions (Barnes, 2002; Rodrigues et al., 1995). Fine roots also store starch. Gholz and Cropper Jr (1991) showed that starch was stored at higher concentrations in fine roots (<5 mm) than in stem tissue in mature slash pine (*P. elliottii* Mill.); the same storage capacity is found in loblolly pine, where Ludovici et al. (2002) found fine roots had more starch storage capacity per weight than the stem component.

Given their importance in water uptake and starch storage, we expected the drought treated seedlings to have proportionally more fine root biomass. However, there are no clear effects of drought on fine root production of pine in the literature. There is mounting evidence that acute drought does not affect biomass allocation to fine roots in southern pine (Hallgren et al., 1991; Hartmann et al., 2013; Kuhns and Gjerstad, 1988; Torreano and Morris, 1998). The lack of differences in fine root partitioning in our study between treatments echoes the results from King et al. (1999) who found that loblolly pine under water stress did not preferentially partition carbon to fine root biomass. In that study, King *et al.* found that greatest fine root partitioning occurred in irrigated treatments. Another study at the stand level found no differences in fine root biomass between wet, ambient, and dry conditions (Joslin et al., 2000). In previous research, drought-stressed loblolly pine reduced the amount of stem biomass partitioning while increasing partitioning to root biomass (Bongarten and Teskey, 1987). However, Bongarten and Teskey did not separate fine and coarse roots. Torreano and Morris (1998) found that drought treatment reduced both stem growth and root growth at similar rates, failing to indicate preferential allocation to below ground components.

A possible explanation for the similarity in fine root biomass partitioning between the well watered and drought treatments comes from Meier and Leuschner (2008) who explained a reduction in fine root biomass in drought-stressed European beech (*Fagus sylvatica* L.) by suggesting that while measurable fine root was diminished, the actual amount of fine root was likely increased due to faster root turnover. The authors suggested that while more carbon was partitioned to fine roots in response to drought, the increased partitioning was not apparent because the fine roots senesced too quickly to be

measured by a single harvest. The most rapid period of fine root decomposition occurs within the first 60 days for genera such as *Fraxinus* and *Larix* (Fan and Guo, 2010). By the time that we harvested seedlings (126 days after drought began), it is possible that some fine root had decomposed and were not included in the fine root sum. However, Hallgren et al. (1991) found no evidence that moisture stress accelerated fine root mortality in loblolly pine seedlings.

Another possibility is that pot confinement may have limited fine root development in our study which may have prevented treatment effects from developing, as has been reported in other southern pine studies (South et al., 2005). However, our pot sizes (6.59 L) were relatively large compared to our seedlings and should not have interfered with biomass growth. For comparison, Bongarten and Teskey (1987) used containers which were one tenth the size of our pots, and reported no problems with root binding in 12 month old loblolly pine seedlings.

Partitioning to coarse root exhibited differences between species as we hypothesized. Shortleaf pine is a species that tolerates fire and drought better than loblolly pine (Lawson, 1990). Increased partitioning to coarse root biomass of shortleaf pine could allow greater non-structural carbohydrate reserves, as coarse root stores the largest pool of carbohydrate in southern pines (Ludovici et al., 2002). Carbohydrate reserves are linked to drought survival (Galiano et al., 2011; McDowell et al., 2011). The greater potential reserve of carbohydrate in shortleaf pine could provide it resources with which to resprout following topkill from fire or to withstand reduced photosynthesis during drought. Loblolly pine's lower partitioning to coarse root suggests that loblolly pine devotes its carbon resources to other biomass components and less to storage.

Hybrid pine's intermediate coarse root likely makes it better suited to tolerate drought than loblolly pine, but it did not have the same relative quantity of coarse root available to store carbohydrate as did shortleaf pine.

As additional benefit of having a larger proportion of coarse root, shortleaf pine may have increased water uptake ability. Macfall et al. (1991) described what they called, "significant regions of water extraction by the taproot" when using magnetic resonance imaging to study the water uptake potential of loblolly pine seedlings in a sand medium. If shortleaf pine has evolved a coarse root system as a mechanism to store starch to survive drought and resprout after fire, it may provide increased water uptake. We could not measure taproot depth or lateral root extension in our pot study. However, if soil exploration is related to coarse root biomass, then shortleaf pine may be able to draw soil moisture from larger soil volume.

A surprising coarse root pattern was lower coarse root biomass partitioning in drought treatment compared to the well watered treatment. The decrease in coarse root in the drought treatment is likely a response to the lower carbon gain in seedlings with reduced photosynthetic activity while seedlings rely on belowground resources for respiration requirements. Hartmann (2013) found no depletion of non-structural carbohydrates (NSC) in the above ground biomass of Scots pine (*Pinus sylvestris* L.) which were exposed to lethal drought stress. However, they reported a significant reduction in NSC in below ground tissue and suggested that root biomass may have actually decreased during the drought. Kuhns and Gjerstad (1988) found that loblolly seedlings exposed to water stress allocated almost no carbon to their root system using  $^{14}\text{C}$  tracing. The authors noted that the proportion of  $^{14}\text{C}$  in sugars actually increased in

the most highly drought stressed seedlings, while other types of carbohydrates had declining  $^{14}\text{C}$  levels, indicating the carbon fixed during drought was being used for respiration in above ground tissue and not stored as starch during drought. If new carbon is being mostly devoted to above ground tissue, belowground biomass will not increase during drought.

Loblolly pine partitioned more biomass to stem than shortleaf or hybrid pine when compared at smaller seedlings size, which indicates that loblolly pine invests more carbohydrate to outgrowing competition aboveground during the first year while shortleaf pine and hybrid invest their carbohydrate to other biomass components, e.g., coarse root. While this difference in stem biomass declined in larger seedlings, it could be an important feature of a young seedling's development. Stem is less efficient than fine and coarse roots at storing NSC in southern pine (Gholz and Cropper Jr, 1991). If drought occurs during the period when loblolly pine is investing a greater proportion of carbohydrate to structural carbohydrate in the stem at the expense of storing NSC reserves (Kuhns and Gjerstad, 1988; Torreano and Morris, 1998), loblolly pine may be at a competitive disadvantage to shortleaf and hybrid pines which have larger coarse root reserves that may allow them to withstand drought. Biomass allocation to stem has been shown to decrease under water stressed conditions (Ledig et al., 1970). However, we found no effects of drought on partitioning to stem in our study.

Partitioning to foliage increased in drought treatments, which was likely an effect of decreased coarse root biomass in drought-stressed seedlings. As partitioning is zero-sum, if coarse root biomass is decreased, biomass must increase in another component and foliage development probably was least affected. Late season drought does not affect

needle biomass with the same intensity as early season drought (Dougherty et al., 1994) because the pine foliage is mostly done growing for the year. We started the drought treatments on September 4th when needle development and height growth had mostly been completed for the year. There was no needle senescence in the time between the study's start and end. As a result, needle biomass mostly reflected the amount prior to drought. Additionally, there is some evidence that foliar NSC are not depleted during drought in Scots pine, reducing the apparent effect on needle biomass (Galiano et al., 2011). There were no species effects on foliage biomass partitioning, which was unexpected. Loblolly pine grows larger when compared to same-aged shortleaf pine. Greater investment to foliage would help explain loblolly pine's accelerated growth and an increase in transpiring surface area may place loblolly pine at a disadvantage when experiencing water stress. However, the similarity in foliage partitioning of loblolly pine and shortleaf pine does not support those hypotheses. Rather than foliage effects, differences between coarse root and stem seemed were the major difference among species.

The lack of differences between species for the soil moisture (FTSW) where species began to reduce transpiration suggests that of the strategies shortleaf pine uses to survive drought don't involve differences in timing of stomatal closure compared to loblolly pine. All three species stopped transpiring at the same soil moisture level, which does not support a hypothesis that shortleaf pine is either: A) more sensitive to water stress and stops transpiring earlier to conserve water, or B) possess adaptations that allow it to continue to transpire well beyond the soil moisture point that loblolly pine must close its stomata. While we found no differences in our southern pine species, other

species do show genetic differences in FTSW values at which transpiration declines. For instance, Jyostna Devi et al. (2009) described wide variation in critical soil moisture when testing peanut plant varieties (*Arachis hypogaea* L.) and Sinclair and Ludlow (1986) were able to compare the water balance of soybean (*Glycine max*), cowpea (*Vigna uniuiculata*), black gram (*Vigna mungo*), and pigeonpea (*Cajanus cajan*).

While others have used FTSW values to identify drought resistant strains, our measured reduction in transpiration at similar FTSW values seems to indicate that altering belowground and aboveground partitioning (coarse root in particular) is the more important life strategy of shortleaf pine in response to drought. Schopmeyer (1939) came to a similar conclusion when comparing loblolly and shortleaf pine transpiration rates across well watered and water stressed treatments. Schopmeyer suggested that the drought-stress advantage shortleaf pine enjoyed was conferred by its high root:shoot values, and not inherent physiochemical differences compared to loblolly pine. Other possible explanations for greater drought tolerance in shortleaf pine could include greater refilling of embolisms after drought, better maintenance of turgor during drought, or advantages defending against biotic attacks during drought stress (Logan et al., 2003; Mattson and Haack, 1987).

Like most other characteristics of the shortleaf x loblolly hybrid pine, biomass partitioning was generally intermediate between the two parent species. The hybrid pine may not grow quite as tall as the loblolly pine, or have the same amount of coarse root as shortleaf pine, but exhibits intermediate growth. This is similar to many other physical traits, such as the intermediate basal crook, needle length, male strobili length, needle



sheath length, needles per fascicle, cone size, bud size, etc (Lilly et al., 2012; Schultz, 1997).

Growth of all three species declined due to drought and there were not differences in the relationship between declining soil moisture and seedling water use, which makes it difficult to determine an ecophysiological mechanism related to differences in drought tolerance between shortleaf and loblolly pine or estimate where hybrid pines fall along the continuum. There may exist differences among species related to drought survival, however, we did not impose drought stress severe enough to kill the seedlings to measure mortality differences. The most noticeable difference was in partitioning to coarse root which, assuming carbohydrate storage and coarse root partitioning are correlated, probably indicates an intermediate ability of the hybrids to withstand drought compared to the more drought tolerant shortleaf pine and less drought tolerant loblolly pine. If so, this may help facilitate the spread of hybrid pines to areas that might otherwise be too xeric for pure loblolly pine.

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**Table 4.1** Arithmetic mean biomass (g) of each seedling component for shortleaf, shortleaf x loblolly hybrid, and loblolly pine seedlings grown under well watered and drought conditions. Standard errors are in parentheses following the means.

	Fine Root	Coarse Root	Stem	Branch	Foliage	Total
Shortleaf Drought	9.15 (1.10)	5.83 (0.69)	2.64 (0.38)	0.91 (0.16)	8.82 (1.07)	27.35 (2.81)
Shortleaf Watered	14.96 (1.64)	13.01 (0.82)	6.32 (0.67)	2.54 (0.20)	16.39 (1.12)	53.22 (2.55)
Hybrid Drought	12.33 (0.70)	6.28 (0.42)	5.08 (0.48)	1.80 (0.19)	17.22 (1.49)	42.71 (2.52)
Hybrid Watered	17.50 (1.30)	10.64 (0.73)	8.77 (0.79)	3.11 (0.19)	20.40 (1.08)	60.42 (3.50)
Loblolly Drought	12.70 (0.72)	5.04 (0.29)	6.31 (0.36)	1.90 (0.25)	16.64 (1.18)	42.59 (2.10)
Loblolly Watered	19.33 (2.00)	9.48 (0.83)	10.63 (1.35)	2.27 (0.33)	19.06 (1.67)	60.77 (5.07)

**Table 4.2** The p values of the Type 3 test of Fixed Effects and slope differences for the relationship between log(total biomass) and each biomass component. If p values of the interaction terms (slopes) were greater than or equal to 0.05, a simplified model (Table 3) was used to evaluate significance of treatments using LSMeans.

Effect	Fine Root	Coarse Root	Stem	Branch	Foliage
Species	0.96	0.37	0.02	0.10	0.74
Log(total)	$\leq 0.0001$	$\leq 0.0001$	$\leq 0.0001$	$\leq 0.0001$	$\leq 0.0001$
Log(total)*species	0.96	0.63	0.02	0.11	0.88
Treatment	0.38	0.98	0.08	0.82	0.27
Log(total)*Treatment	0.37	0.72	0.07	0.85	0.15
Species*Treatment	0.23	0.16	0.22	0.32	0.21
Log(tot)*Species*Treat	0.25	0.16	0.21	0.27	0.21

**Table 4.3** The p values of the model without slopes from the test of log(biomass component) and log(total biomass) against species and treatment. Stem was not tested because of the statistically slope differences among species for the relationship between log(stem) and log(total). Any p values greater than or equal to 0.05 were not considered statistically significant.

Effect	Fine Root	Coarse Root	Stem	Branch	Foliage
Species	0.55	$\leq 0.0001$	NA	0.13	0.06
Log(total)	$\leq 0.0001$	$\leq 0.0001$	NA	$\leq 0.0001$	$\leq 0.0001$
Treatment	0.62	$\leq 0.0001$	NA	0.99	$\leq 0.0001$
Species*Treat	0.28	0.48	NA	$< 0.01$	0.09



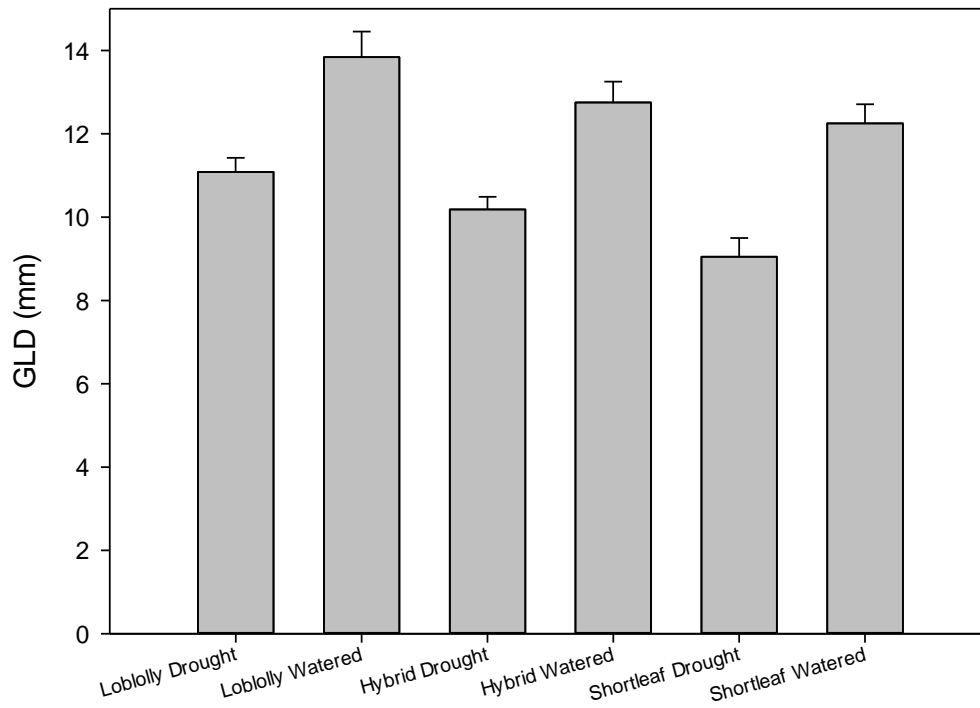
**Table 4.4** LSMeans estimates of the transformed data in log(grams).

	Fine Root	Coarse Root	Stem	Branch*	Foliage
Shortleaf Drought	2.63	2.20	1.65	1.07	2.71
Shortleaf Watered	2.57	2.43	1.55	1.13	2.62
Hybrid Drought	2.57	1.88	1.69	1.07	2.89
Hybrid Watered	2.61	2.11	1.77	1.19	2.72
Loblolly Drought	2.60	1.68	1.86	1.10	2.88
Loblolly Watered	2.71	1.99	2.00	0.92	2.66

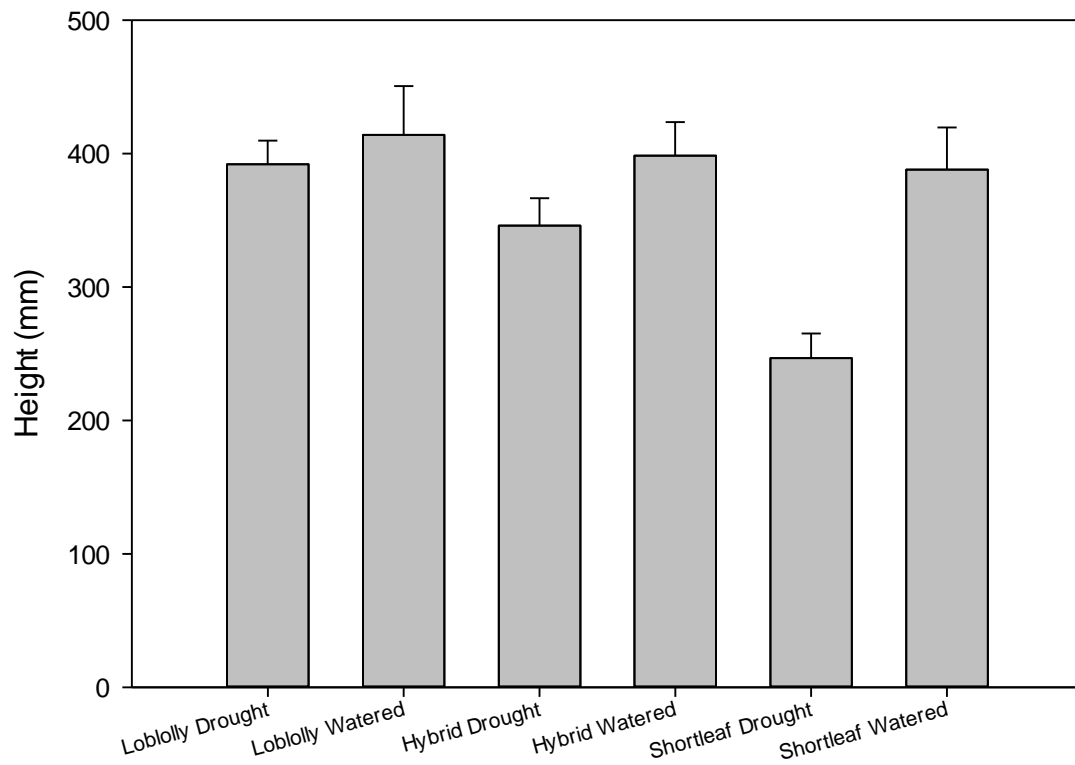
\*branch weights were sometimes less than 1.0 g, means of Branch were calculated as  $\log(1+\text{branch})$

**Table 4.5** Stem regression equations for species from log transformed data. Loblolly pine's slope is less steep than shortleaf and hybrid pines. Stem biomass at 1.5 and 1.8 log(total) (g) are calculated for comparison.

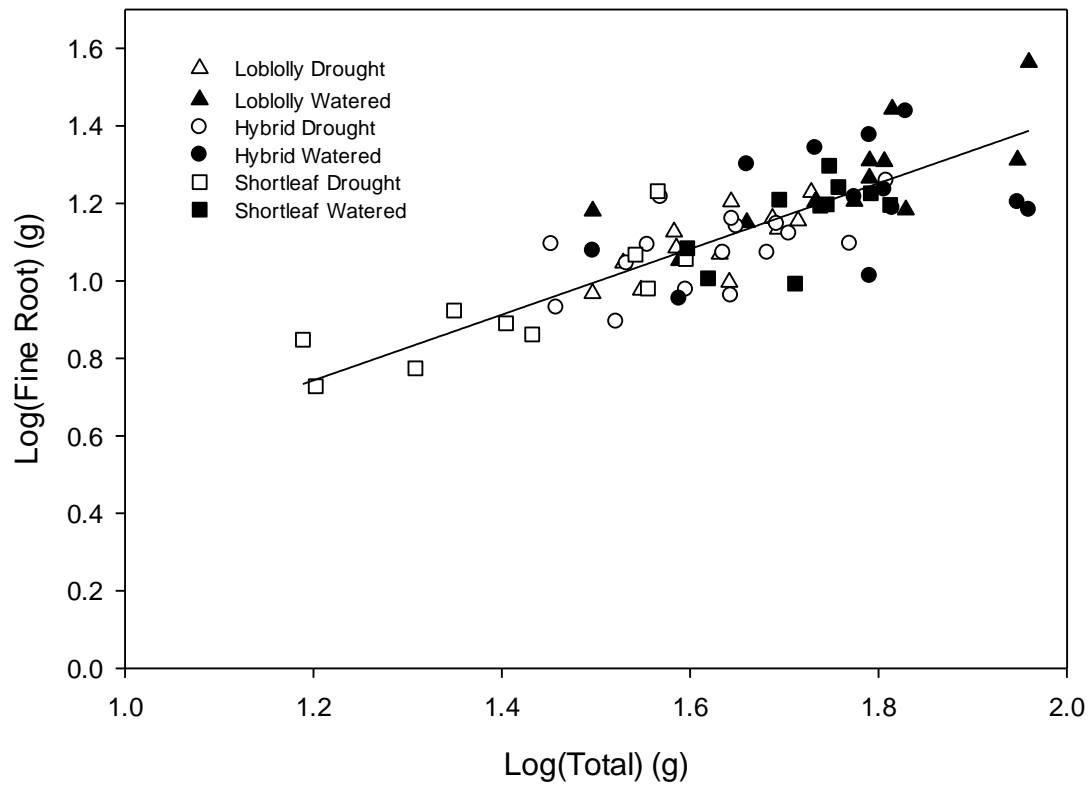
	Regression Equation	$r^2$	Stem biomass at 1.5 g total	Stem biomass at 1.8 g total
Shortleaf	$\hat{y} = -1.5617 + 1.3641x$	0.9269	0.48	0.89
Hybrid	$\hat{y} = -1.6722 + 1.4559x$	0.8055	0.51	0.95
Loblolly	$\hat{y} = -0.9832 + 1.1061x$	0.6621	0.68	1.01



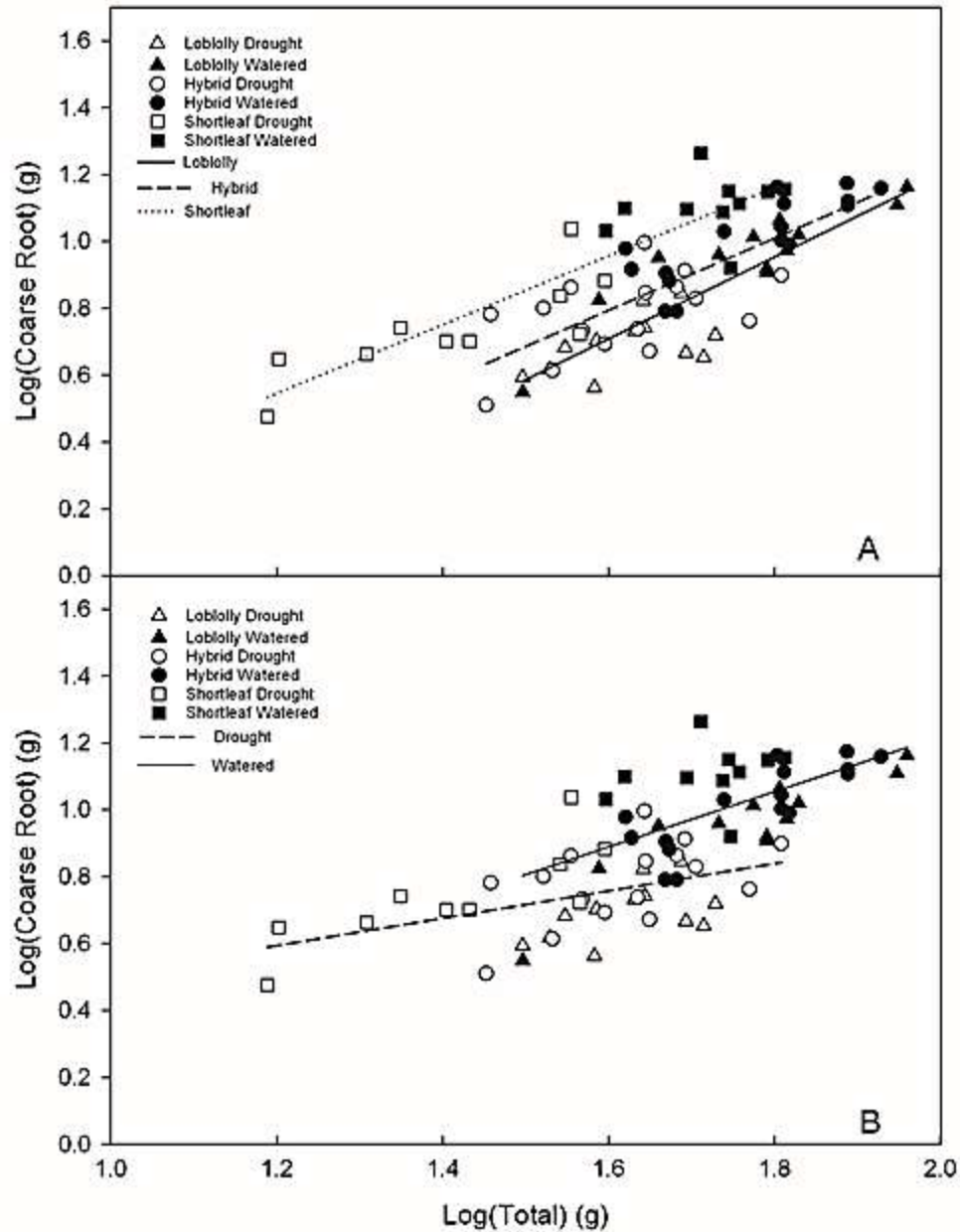
**Figure 4.1** Ground line diameter of one-year-old loblolly, shortleaf x loblolly hybrid, and loblolly pine seedlings growing under well watered or drought conditions.



**Figure 4.2** Heights of one-year-old, shortleaf, shortleaf x loblolly hybrid, and loblolly pine seedlings growing under well watered or drought conditions.



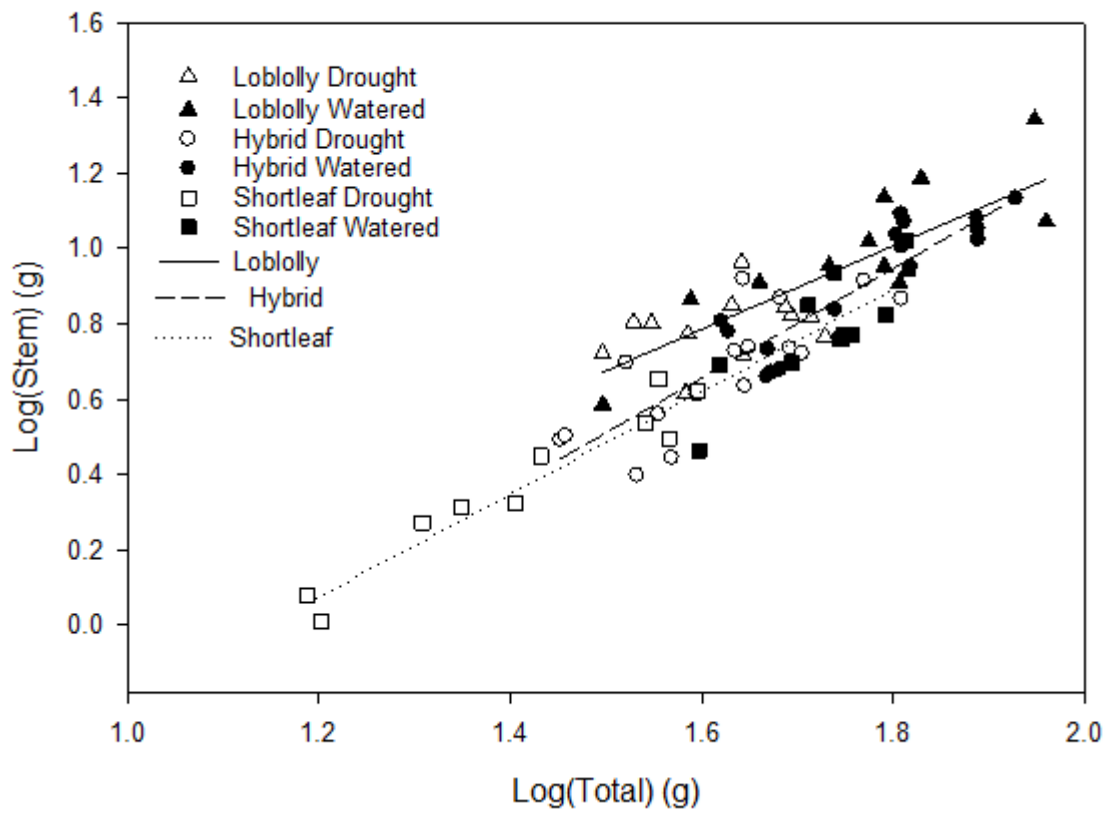
**Figure 4.3** Relationship between log(fine root biomass) and log(total seedling biomass) for well watered and drought stressed seedlings of shortleaf, hybrid, and loblolly pine seedlings. No species or treatment effects were significant



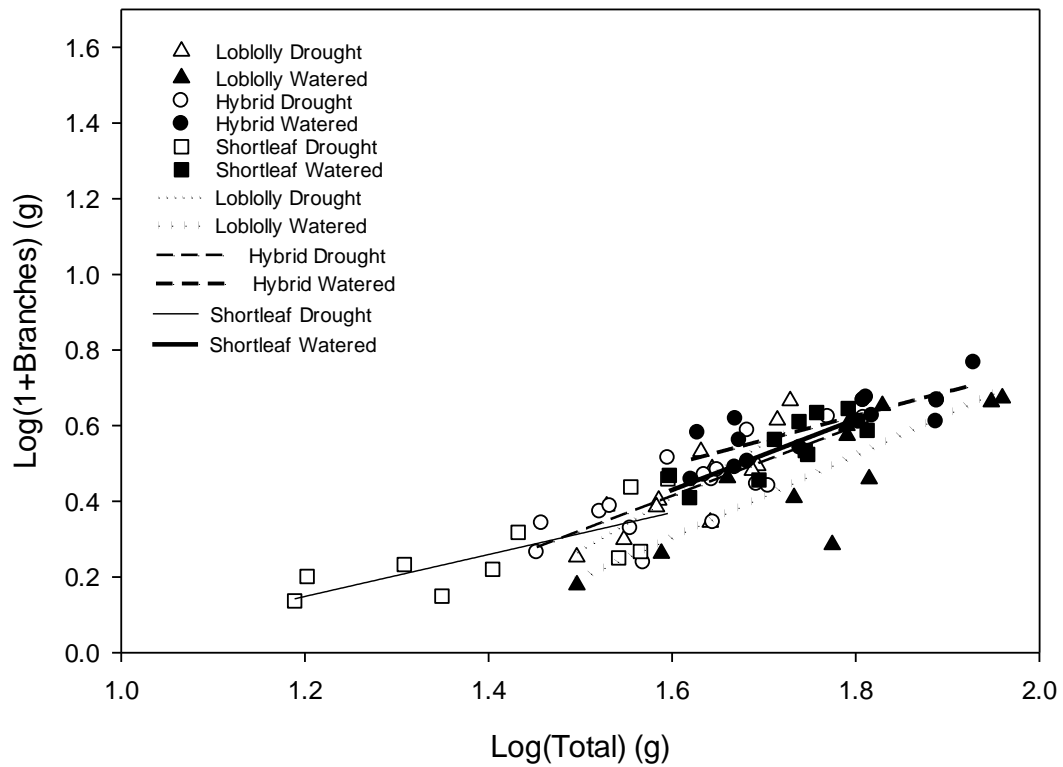
**Figure 4.4**

**A** Relationship between  $\log(\text{coarse root biomass})$  and  $\log(\text{total seedling biomass})$  for well watered and drought stressed seedlings of shortleaf, hybrid, and loblolly pine seedlings. Shortleaf pine seedlings had more coarse root than hybrid pine seedlings, which had more coarse root than loblolly pine seedlings.

**B** Coarse root biomass and the effect of water. Slopes of the watered treatment and drought treatment were N.S. Watered seedlings had significantly greater coarse root biomass across all seedling sizes.

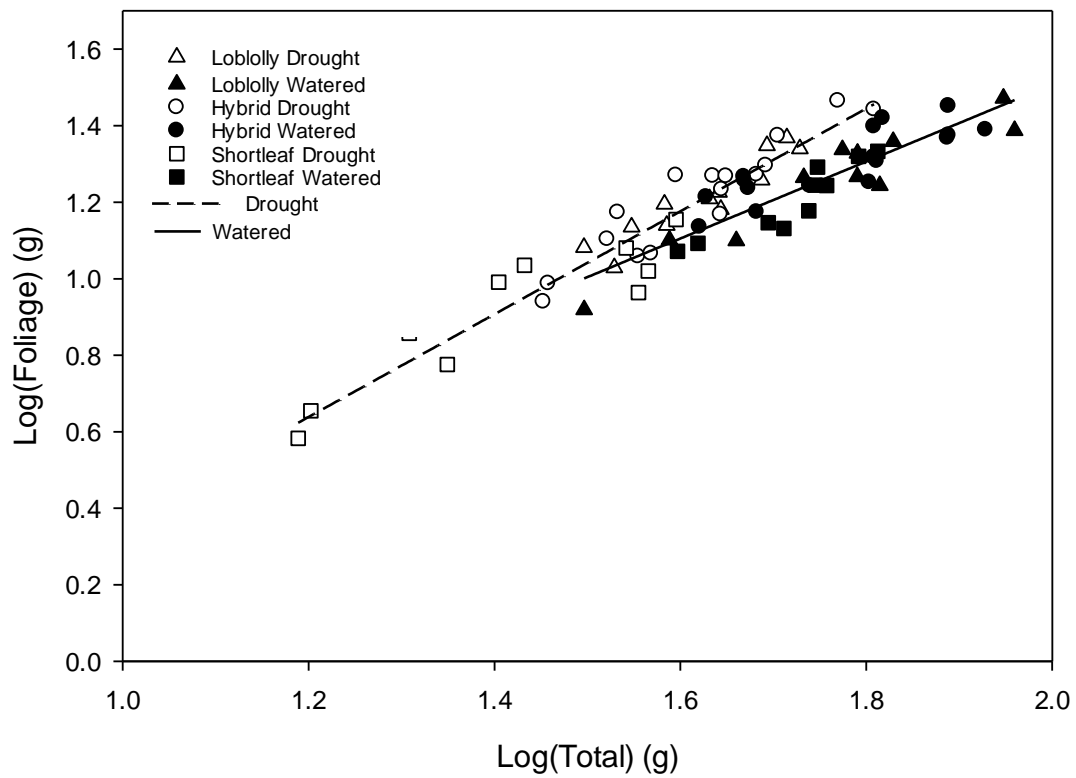


**Figure 4.5** Stem biomass and the effect of species. There was an interaction between log(total) and species; loblolly pine had greater stem biomass at lower total weight, but at higher total weight, shortleaf and hybrid pines' stem biomass approached loblolly pine's mass.

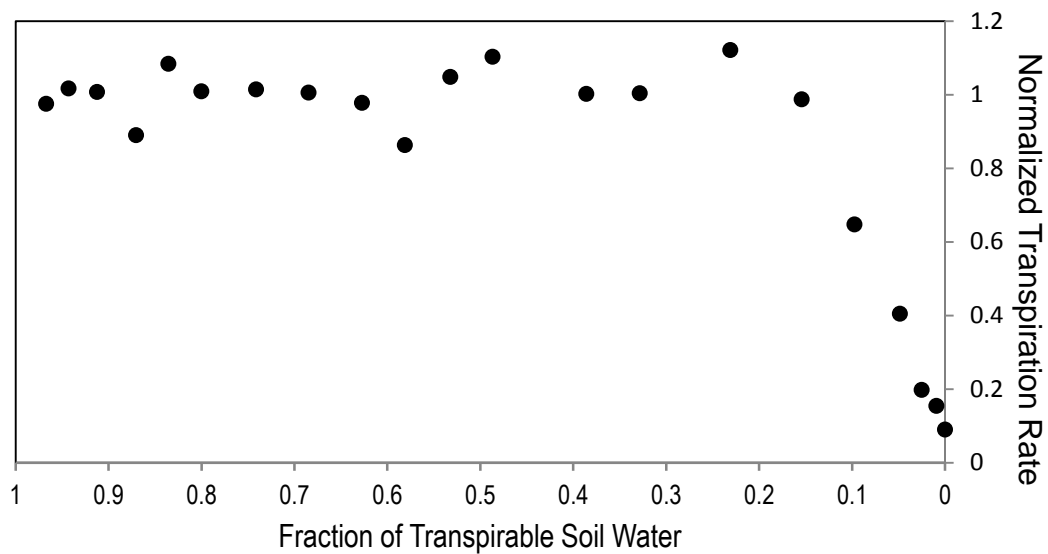


**Fig 4.6** Branch biomass and the effects of species and treatment. There was a significant interaction between species and treatment, preventing interpretation.





**Figure 4.7** Foliage biomass and the effect of water. Foliage biomass LSMeans were higher in drought seedlings than in watered seedlings.



**Figure 4.8** Relative transpiration rates plotted against soil moisture (NTR vs FTSW). This is a representative graph from a loblolly pine seedling.

## CHAPTER V

*Formatted for Trees - Structure and Function*

### **Shortleaf x Loblolly Hybrid Pine Seedlings Do Not Resprout Following Topkill from Fire**

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#### **Abstract:**

Shortleaf pine (*Pinus echinata* Mill.) is an ecologically important species in the southern forest of the United States that is fire adapted. Hybridization between shortleaf and loblolly pines (*Pinus taeda* L.) has increased since the 1950s, possibly reducing the resilience of pine forests formerly composed of fire adapted shortleaf pine. Specifically, shortleaf x loblolly hybrid pine seedlings may not be as fire tolerant as shortleaf pine seedlings because they lack a strong basal crook which facilitates shortleaf pine seedlings to resprout after fire by keeping dormant buds near the soil surface. We planted shortleaf pine, loblolly pine, and shortleaf x loblolly hybrid pine seeds near Idabel, OK, USA.

Seedlings were allowed to grow for 12 or 16 months before being treated with a dormant season (March) or growing season (August) burn. In the dormant season burn, a layer of wetted duff was added to half of the seedlings from all species. Seedling size, basal crook presence, and maximum fire temperature were recorded. We found that after 12 and 16 months, shortleaf pine seedlings possessed strong basal crooks while hybrid pines expressed weak basal crooks. Shortleaf pine survived fire in the dormant season (43%) and growing season (56%). Hybrid pine had one surviving seedling from the dormant season burn and one surviving seedling from the growing season burn. No loblolly pine seedlings survived either burn. We conclude that the weak basal crook of the hybrid pine does not protect the dormant buds from fire, unlike shortleaf pine's strong basal crook. With increasing hybridization across the southern United States, land managers can use fire to remove hybrid pines seedlings from shortleaf pine forests. Eliminating hybrid pines will both preserve the genetic integrity of the shortleaf pine population and also select for more resilient pines in a future where projections forecast more frequent fire activity.

**Keywords:** shortleaf pine, loblolly pine, hybrid, fire, basal crook

**Key Message:** The weak basal crook of the shortleaf x loblolly hybrid pine seedling fails to protect it from fire whereas shortleaf pine seedlings' strong basal crook enables resprouting after topkill

## Introduction:

The southeastern pine forests of the United States have historically coexisted with frequent fire disturbance (Komarek, 1974). Shortleaf pine (*Pinus echinata* Mill.), which once dominated much of the southeastern United States, has evolved to protect itself from fire by possessing higher resprouting capacity than related southern pines (e.g. loblolly pine (*Pinus taeda* L.)) (Will et al., 2013). Shortleaf pine also resprouts more quickly after topkill than loblolly pine (Liu et al., 2011). Perhaps shortleaf pine's most important adaptation to fire is the basal crook, first described by Mattoon (1915). The basal crook is a section of stem which is bent horizontally along the ground, pressing the dormant buds located in the axils of the primary needles against the ground (Stone and Stone, 1954). The basal crook protects the dormant buds from being exposed to fire, allowing the seedling to resprout after topkill. The basal crook usually forms in the first or second year and persists until the seedling is about 10 years old (Little and Somes, 1956). Once a dominant southeastern forest component, shortleaf pine has recently suffered a 52% decline (Oswalt, 2011). This decline can be partially attributed to land use change, replacing shortleaf forests with loblolly pine plantations, and fire exclusion. Furthermore, the number of shortleaf x loblolly hybrid pines in the shortleaf population has surged in the past 60 years from 3% to 46%, threatening shortleaf pine with extinction by introgression (Stewart et al., 2012; Tauer et al., 2007).

Shortleaf x loblolly pine hybrids have likely always occurred as a small percentage of the southern pine component (e.g. Cotton et al, 1975). The cause of the recent rapid increase in the number of shortleaf x loblolly pine hybrids is not known. Hybridization was historically thought to occur rarely, due to "freak" weather (Zobel,

1953) that caused overlap in pollen shed (loblolly pine typically sheds pollen before shortleaf pine). The long term exclusion of fire has caused dramatic changes in the species composition of the southeastern forest (Nowacki and Abrams, 2008) and could be driving the success of the hybrid pine. Stewart et al. (2014) found that frequent fire excluded hybrid and loblolly pine seedlings from a mixed shortleaf-loblolly pine forest while adjacent unburned stands had a seedling population composed of loblolly, shortleaf, and hybrid pines. Likely a cause of their fire intolerance, Lilly et al. (2012) found that hybrid pine seedlings only weakly expressed the protective basal crook which shortleaf pine seedlings develop to survive topkill by fire. The weak crook of the hybrid pines consists of a bend with an angle of approximately 45° which does not lower the dormant buds to the surface of the soil.

The southeastern United States will likely experience an increase in fire activity in the future (Flannigan et al., 2000). While hybrid pine grow faster than shortleaf pine, they do not have the same fire tolerant characteristics that have allowed shortleaf pine to become a stable component of the historic southeastern forest (Lilly et al., 2012; Will et al., 2013). Previous work looking at hybrid pine fire tolerance tested hybrid pine's resprouting after topclipping and girdling with a propane torch (Will et al., 2013). While a satisfactory test of the hybrid's resprouting potential, girdling with a propane torch failed to test the effect of the basal crook or include the effects of burning the crown. In a wildfire, the basal crook protects the shortleaf pine's dormant buds from fire. The hybrid's weak basal crook may not afford it any protection from fire, which would make hybrid pine as susceptible to fire as the fire intolerant loblolly pine. However, it is not known if the hybrid's basal crook provides any protection.

Presently, the hybrid swarm is ascendant, representing increasing numbers of stems in the southeastern forest and also representing a larger genetic portion of the shortleaf pine population. If the once fire-adapted shortleaf pine forest becomes increasingly composed of less fire resistant pine (i.e. the hybrid pine), then the southeastern forest is at risk in a future of increasing fire activity.

We sought to test the hybrid pine's fire tolerance and compare it to the fire tolerance of shortleaf pine and the fire intolerant loblolly pine. Determining crook traits, fire intensity, and survival after controlled burns, we hypothesized that a) shortleaf pine would exhibit stronger crooking behavior, while hybrid pine would express only weak crooks, and b) that shortleaf pine would successfully survive topkill by fire and resprout, while hybrid and loblolly pines would fail to resprout following fire.

**Methods:**

Shortleaf pine, loblolly pine, and shortleaf x loblolly pine seeds were produced at the Oklahoma Forestry Services seed orchard in Idabel, Oklahoma, USA (33.893970, -94.751623). Both shortleaf pine and loblolly pine seeds were collected from open pollinated cones from six shortleaf pines and six loblolly pines. Hybrid pine seeds were created through controlled pollination of six loblolly and five shortleaf parent trees. Each parent tree was used in one or two crosses to make eight total hybrid families. Shortleaf pine pollen was used to fertilize loblolly pine. Seeds were collected from cones and stored in a -20 °C freezer.

On 24 January 2013, seeds were removed from the freezer and soaked in water for 12 hours at approximately 4 °C. Seeds that sank were selected for stratification, while floating seeds were discarded. Seeds were then stratified in a refrigerator wrapped in a moist paper towels contained in a plastic bag, at 4 °C for 56 days.

Seeds were planted at the Kiamichi Research Station, Idabel, Oklahoma on 28 March 2013. The field was approximately 0.2 ha, and had been tilled two months prior to planting using tractor mounted tiller to a depth of 45 cm. The soil is 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 percent. Seeds were planted in 14 rows, each row containing a random order of six loblolly and six shortleaf (one from each half-sib family) as well as twelve hybrid pines (one from each full-sib family). The number of planting sites totaled 280 (20 in each row x 14 rows). In each planting site, five to ten seeds were covered by 1 to 2 cm of mineral



soil. Seedlings were marked by numbered, metal tags attached to stakes. Chicken-wire enclosures were placed over each planting site and staked into the ground.

By 13 April 2013, not all planting sites had identifiable seedlings. Additional seeds were planted into 50 mL of prepared soil in Leach tubes (Stuewe and Sons, OR, USA) at a greenhouse in Stillwater, OK on 15 April 2013. Seeds had been cold stratified for 80 days. The additional seeds were planted in a mixture of: three parts soil from the field site, two parts coarse sand, and one part peat moss. These additional seeds were watered and kept at a daytime temperature of 24 °C and a nighttime temperature of 20 °C. On 3 May 2013, approximately 100 unsuccessful planting sites were transplanted with the additional seedlings of the original genotype. Seedlings were planted such that the root collar of the seedling was at the soil surface after transplanting. Before transplanting, seedlings were removed from the soil mixture in which they had been growing and planted directly into the soil, with all bare roots carefully covered by the soil.

Throughout the summer of 2013, the seedlings were watered, weeded, and fertilized. Water was applied using overhead sprinklers as needed with the goal to keep the seedlings alive and actively growing. Competition control consisted of directed sprays of glyphosate while covering seedlings with a protective canister, and hand weeding of competition too close for chemical application. On 25 June 2013 the planting sites were thinned to leave one to three seedlings. Multiple seedlings were left if more than one existed and if they were separated by more than 5 cm.

On 16 March 2014, one third of 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), number of flushes, and height to location of

primary needles (nearest mm) were measured. Primary needles were no longer identifiable by August 2014, so primary needle height is only available for the March burn.

Half the rows were selected to receive a layer of duff with the remaining half not receiving 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. Duff treatment consisted of adding approximately 1 cm of duff in a 10 cm diameter circle surrounding the seedling. Duff was moistened needles and leaves that had decomposed on the forest floor for greater than one year in a nearby mixed pine-hardwood stand.

On 17 and 18 March 2014, selected seedlings were burned between 1100 and 1800 hours. Burning the seedlings consisted of piling 1.0-1.4 kg of air-dried loblolly pine litter in a 0.5 m<sup>2</sup>, 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.

To quantify fire intensity, type K thermocouples were placed at ground level next to the dormant buds, 10 cm above the ground, and 20 cm above the ground. Temperatures were recorded every 0.5 second. Fires were started at the downwind edge of the circle with an accelerant and moved through the plots as a backfire, lasting about six minutes.

Weather conditions on 17 March averaged 10.4 °C (ranged from 7.4 to 12.6 °C), relative humidity averaged 64% (ranged from 57 to 71%), and wind speed averaged 2.0 m s<sup>-1</sup> (ranged from 0.7 to 4.2 m s<sup>-1</sup>). On 18 March, temperatures averaged 12.6 °C (ranged from 5.5 to 21.2 °C), relative humidity averaged 67% (ranged from 41% to 90%), and wind speed averaged 6.1 m s<sup>-1</sup> (ranged from 3.1 to 9.0 m s<sup>-1</sup>). 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.

Approximately half of the remaining two-thirds of the living seedlings were burned (26 loblolly pines, 26 hybrid pines, and 16 shortleaf pines). Ambient temperature during the 25 August burn averaged 33.6 °C (ranged from 32.4 to 34.8 °C), relative humidity averaged 48% (ranged from 42 to 57%), and wind speed averaged 1.6 m s<sup>-1</sup> (ranged from 0.4 to 3.2 m s<sup>-1</sup>). On 26 August, ambient temperature averaged 28.1 °C (ranged from 22.8 to 33.5 °C), relative humidity averaged 71% (ranged from 52 to 92%), and wind speed averaged 7.4 m s<sup>-1</sup> (ranged from 3.1 to 9.6 m s<sup>-1</sup>). Weather conditions were measured at the Idabel Weather Station (Oklahoma Mesonet, Oklahoma Climatological Survey). The August burn closely followed the methodology of the first burn. Some slight modifications to the initial burn treatments were made and are as follow: 1.4 kg of pine straw was added around the seedlings, duff was not added to any seedlings, and thermocouples were only placed at ground level. Location of the primary needles could no longer be determined and was not measured. Seedlings were examined for survival and resprouting 35 days later on 30 September 2014.

Thermocouple data was screened for missing and erroneous data. Maximum temperatures were calculated for each planting site burn. Proc GLM was used to test for differences in fire temperatures among species in SAS 9.3.

## Results

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. Species had a marginally significant effect ( $p = 0.07$ ) on height with loblolly pine greater than shortleaf pine (Table 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.

Of the 23 shortleaf pine seedlings burned in March, 10 survived (43%). Of the 33 hybrid pine seedlings burned, one survived (3%). Of the 26 loblolly pine seedlings burned, none survived (Table 2). All but two shortleaf pine seedlings had strong basal crooks (Table 2). No hybrid pine seedlings had strong basal crooks. The majority of hybrid pine seedlings had weak crooks (67%). 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.

Height to the primary needles was significantly influenced by species ( $p < 0.001$ ). Primary needles were lower in shortleaf pine ( $8.5 \pm 1.8$  SE mm) than in hybrid pine ( $20 \pm 1.4$  SE mm) ( $p < 0.001$ ), and hybrid pine had lower primary needles than loblolly pine ( $35 \pm 1.6$  SE mm) ( $p < 0.001$ ). The sole resprouting hybrid pine's primary needles were 12 mm above the ground.

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 ( $424^{\circ}\text{C} \pm 41$  SE)

than in plots with duff ( $180^{\circ}\text{C} \pm 38\text{ SE}$ ) ( $p < 0.0001$ ) (Table 2). There were differences in temperatures for species ( $p = 0.005$ ), with shortleaf pine ( $206^{\circ}\text{C} \pm 49\text{ SE}$ ) and hybrid pine ( $285^{\circ}\text{C} \pm 44\text{ SE}$ ) experiencing lower temperatures than loblolly pine ( $423^{\circ}\text{C} \pm 44\text{ SE}$ ). Only shortleaf pine seedlings had enough resprouting seedlings to compare the effect of duff and. no duff. Adding duff had significant effect by increasing shortleaf pine seedling resprouting ( $p = 0.04$ ) with 70 percent resprouting with duff and 30 percent resprouting without duff treatment.

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) (Table 5.1). Regardless of seedling size, the 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%) (Table 3). Of the 26 hybrid pine seedlings, four had no basal crook (15%), 22 had a weak crook (85%), and no 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 lone resprouting hybrid pine had a weak basal crook.

August burns were hotter in shortleaf and loblolly pine ( $557^{\circ}\text{C} \pm 32\text{ SE}$  and  $529^{\circ}\text{C} \pm 41\text{ SE}$ , respectively) seedlings than in hybrid pine seedlings ( $429^{\circ}\text{C} \pm 58\text{ SE}$ ) ( $p = 0.002$ ).

## Discussion

The general trend of seedling growth in our study followed the trend previously reported for shortleaf, shortleaf x loblolly hybrid pine, and loblolly pines (Lilly et al., 2012; Will et al., 2013). The largest seedlings in the field were predictably loblolly pine or hybrid pine seedlings, while the smallest seedlings were typically shortleaf pine seedlings. There was, however, more variation in seedling size than in studies with more controlled conditions (Will et al. 2013, Bradley et al. 2015). This was likely due to much greater variation in edaphic and hydrologic conditions among the planting sites in the field.

The addition of duff to the March burn treatment increased shortleaf pine seedlings' success in resprouting. Mattoon (1915) noted that the basal crook of the shortleaf pine could often become covered with soil and duff, thus adding a layer of insulation between the dormant buds in the crook and the heat of a fire. This insulating effect of the duff treatment is evident in the temperature data, where temperature at the level of the dormant buds of shortleaf, hybrid, and loblolly pines was lower in the duff treatment than in the no duff treatment. Despite the protection and lower temperatures afforded by the addition of a duff layer, only one hybrid pine resprouted following the March burn. The duff layer was only effective when paired with the strong basal crook of shortleaf pine (70% of duff treated shortleaf pine resprouted). The addition of approximately a 1 cm deep duff layer was adequate to cover most of the shortleaf pine seedlings' basal crooks and dormant buds. However hybrid and loblolly pine held their dormant buds (in the axils of primary needles) typically above the duff layer. We chose not to apply a duff layer during the August burn because the seedlings were larger and

because we had shown that shortleaf pine had the capability to sprout without duff added. As such, our results from the August burn likely underestimate survival from a situation where shortleaf pine is regenerating in areas with a developed duff layer.

Although loblolly pine and shortleaf x loblolly hybrid pine seedlings were generally larger, shortleaf pine composed the majority of resprouting in both the March burn and August burn (only one hybrid pine resprouted following each burn). This is likely due to the strong expression of the basal crook in the shortleaf pine seedlings. Our results again confirm that the hybrid pine has a weakly expressed basal crook compared to shortleaf pine (Lilly et al., 2012; Will et al., 2013), which contributes to why shortleaf pine had far higher resprouting success than the weakly crooking hybrid pine. Loblolly pine, which lacks a basal crook, did not resprout. When examining the seedlings for resprouting, new shoots were typically resprouting along the length of the basal crook, rather than the base of the stem (Figure 5.1).

The main function of the basal crook in regards to surviving fire appears to be that it lowers the dormant buds to the soil surface, protecting the dormant buds from fire. When the crook is in contact with the ground the dormant buds on the underside of the crook are protected. Most shortleaf pine with a strong crook had their dormant buds in contact with the soil. Even in the case of a basal crook above the soil surface, the dormant buds were lower than the dormant buds of seedlings with weakly expressed basal crooks or seedlings with no basal crook. Our measurements of height to the primary needles during the March burn were taken from the middle of the horizontal section of the basal crook such that half the basal crook was below the measured height, and some dormant buds were often in contact with the soil (Figure 5.2). Those shortleaf pine that lacked the

formation of a strong basal crook had primary needles near the soil surface. When a seedling develops so that dormant buds are near the soil surface (or is planted deep to protect the dormant buds), a crook may not form due to the inability to 'bend' over. However, in these cases, a crook is not necessary for resprouting because the dormant buds are already protected by the soil surface.

Shortleaf pine seedlings kept their dormant buds (quantified by the measuring the location of the primary needles) lower than the other two species, averaging 8.6 mm. The hybrid that resprouted in March had primary needles only 12 mm above the soil surface. Compared to the hybrid pine average of 19 mm, the surviving hybrid pine's dormant buds were very close to the soil surface which could have contributed to its success. Loblolly pine elevated its dormant buds 35 mm above the soil, leaving them completely exposed to fire. Reasons for variation in height to primary needles of non-crooking seedlings could be related to planting depth, growth rate, genetics, soil conditions, soil fauna, etc. However, only a small number of hybrid pine had dormant buds at a height similar to shortleaf pine and none had dormant buds pressed in contact with the mineral soil. March and August burns both had identical fuels type with similar fuel loads. Despite this, average temperatures were higher in the August burn - likely a consequence of the summer's higher ambient temperatures and lower soil moisture. While Shelton and Cain (2002) reported drastic differences in shortleaf pine seedling survival rates between winter and summer burns, i.e. near complete survival of winter burn, and no resprout in summer burns, we did not find large differences in resprouting rates between March (43%) and August burns (56%) for shortleaf pine seedlings.



Fire has historically shaped the southern United States, creating conditions favorable for the perpetuation of southern pine forests (Vose et al., 1995). The rapid increase of the hybrid pine component in the last 60 years (Stewart et al., 2012) is likely partially due to the exclusion of fire. We show that fire kills shortleaf x loblolly pine hybrid and loblolly pine seedlings. Repeated fires over time can shift the seedlings population. Stewart et al. (2014) showed that a fire return interval of 2 years was capable of selecting against hybrid pine seedlings under a loblolly-shortleaf-hybrid canopy. Meanwhile, in neighboring unburned plots, loblolly and hybrid pines were far more frequent than in the adjacent burned areas.

As wildfire activity may increase across the southern United States (Flannigan et al., 2000; Liu et al., 2010), it is critical that our forests be composed of resilient and fire adapted species. Since Mattoon's 1915 survey, the shortleaf pine forest has been in decline. The threat of introgression by loblolly pine (Stewart et al. 2012) is yet another pressure on a species at risk (Tauer et al., 2007; Oswalt, 2011). The return of frequent fire to the southern forest will promote the natural regeneration of fire adapted species like shortleaf pine (e.g. Williams, 1998). We have demonstrated that fire will help eliminate hybrid pine seedlings, protecting shortleaf pine forests from further introgression and increasing the resilience of the southern forest to fire.

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**Table 5.1** Ground line diameter (GLD) and height of burned seedlings in both March and August, 2014

	<b>March 2014</b>		<b>August 2014</b>	
	<b>GLD (mm) <math>\pm</math>SE</b>	<b>Height (cm) <math>\pm</math>SE</b>	<b>GLD (mm) <math>\pm</math>SE</b>	<b>Height (mm) <math>\pm</math>SE</b>
Shortleaf	5.0 $\pm$ 0.6	13.9 $\pm$ 2.1	16.3 $\pm$ 1.9	66.2 $\pm$ 6.8
Hybrid	5.1 $\pm$ 0.5	16.5 $\pm$ 1.6	25.0 $\pm$ 4.2	81.0 $\pm$ 6.9
Loblolly	6.4 $\pm$ 0.5	19.9 $\pm$ 1.5	20.3 $\pm$ 2.1	83.3 $\pm$ 6.6

**Table 5.2** March burn crooking, fire temperature, and resprouting of shortleaf, shortleaf x loblolly pine hybrids, and loblolly pine seedlings. Duff was added to approximately half the seedlings before burning.

<b>Species</b>	<b>Resprout/Dead</b>	<b>No/Weak/Strong Burned</b>	<b>No/Weak/Strong Survival</b>	<b>Max Temp</b>
Shortleaf	10/13	2/0/21	1/0/9	210 °C ±41
Duff	7/3	0/0/10	0/0/7	121 °C ±60
No Duff	3/10	2/0/9	1/0/2	290 °C ±87
Hybrid	1/32	11/22/0	1/0/0	282 °C ±50
Duff	1/13	5/9/0	1/0/0	137 °C ±38
No Duff	0/19	6/13/0	0/0/0	433 °C ±70
Loblolly	0/26	22/4/0	0/0/0	413 °C ±59
Duff	0/12	10/2/0	0/0/0	296 °C ±80
No Duff	0/14	12/2/0	0/0/0	550 °C ±68

**Table 5.3** August burn crooking, fire temperature, and resprouting of shortleaf, shortleaf x loblolly pine hybrids, and loblolly pine seedlings.

	<b>Resprout/Dead</b>	<b>No/Weak/Strong Burned</b>	<b>No/Weak/Strong Resprout</b>	<b>Max Temp</b>
Shortleaf	9/7	1/3/12	0/2/7	557 °C ±32
Hybrid	1/25	4/22/0	0/1/0	429 °C ±58
Loblolly	0/26	26/0/0	0/0/0	529 °C ±41



**Figure 5.1** A small resprouting shortleaf pine seedling after the March 2014 burn near Idabel, Oklahoma. The seedling was completely immolated. Sprouts originated from a strong basal crook, which is buried under the soil surface.



**Figure 5.2** Resprouting shortleaf pine seedlings from the August 2014 burn near Idabel, Oklahoma. The left seedling's basal crook is almost completely buried by soil, with just a small section exposed, visible above the soil surface.



## CHAPTER VI

### Conclusion

Shortleaf pine is adapted to fire and drought - it has persisted with these disturbances while expanding to eventually become the southern pine with the largest range in the United States. During that time of range expansion, drought and fire were more common than they were in the 20th century (Cook et al., 2007; Fowler and Konopik, 2007). Shortleaf pine was dominant across much of the southeastern United States, making it reasonable to surmise shortleaf pine is well suited to tolerate those disturbances. The historic presence of shortleaf pine in the southern forest added a degree of resilience and hardiness that is currently being lost due to its decline. Once the dominant southern pine, shortleaf pine is now in decline in part because of habitat fragmentation, land use change, climate change, fire exclusion, and introgression.

The southern United States enjoyed higher than average rainfall in the 20th century (Frich et al., 2002), possibly reducing the competitive advantage that shortleaf

pine possesses over faster growing but less drought tolerant species like loblolly pine. Even more disruptive was the suppression and exclusion of fire in the southern timber lands (Nowacki and Abrams, 2008). Shortleaf pine could not rely on its fire tolerance to give it an advantage when competing with hardwood seedlings and faster growing pines. The fire sensitive species that established during fire exclusion interrupted the disturbance regime that encouraged the return of fire, creating a positive feedback cycle of mesophication. During this time, hybrid pine seedlings have been able to persist and grow in number, becoming established in the canopy of some stands.

My results emphasize the necessity of a return of disturbance to restore and conserve shortleaf pine. With increasing numbers of hybrid pines and loblolly pines through shortleaf pine's range in the southern United States, the forest-level tolerance to drought or fire may be compromised. While the hybrid pine appears to have intermediate drought tolerance characteristics (e.g. allometry), I demonstrated that topkilled hybrid pine seedlings were unable to resprout after fire. A hybrid pine forest disturbed by wildfire may not recover, or may recover slowly; in contrast, shortleaf pine forests has thrived for centuries in the presence of frequent fire (Mattoon, 1915; Stambaugh et al., 2007). It is likely that the future southern pine forest will be challenged by greater water stress (Romero-Lanko et al, 2014) and more frequent fire (Flannigan et al., 2000; Liu et al. 2010) than have occurred in the last century. Maintaining pure shortleaf pine should be a goal for natural resource managers across the south who seek to promote stability and resilience in their forests.

In order to restore and maintain shortleaf pine forests, disturbance will need to be carefully reintroduced until disturbance frequencies approach historic norms. Drought would be a difficult disturbance for managers to impose, it would be unlikely to have much of an immediate effect on the hybrid pine population, and it would have deleterious effects on shortleaf pine regeneration, seedlings, and seed sources. Alternatively, prescribed burning comes with many benefits. Prescribed burns prepare the seedbed for seed fall and germination (Yocum and Lawson, 1977). Prescribed burns eliminate seedling and sapling competitors of shortleaf pine (Williams, 1998). Prescribed burns increase biodiversity, habitat quality, and shift the vegetation composition to species that enable the spread and return of wildfire, breaking the cycle of mesophication (Blair and Feduccia, 1977; Nowacki and Abrams, 2008). Perhaps most crucially, the current swell of the hybrid pine seedlings would not survive the reintroduction of wildfire. Now is a critical time for land managers to burn off hybrid pine seedlings before the majority reach sizes large enough to escape the effect of fire and become entrenched in the forest canopy.

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