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Testing the reliability of morphological patterns to identify Sonderegger pine in forest tree seedling nurseries

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Abstract

Sonderegger pine (*Pinus* x *sondereggeri* H.H. Chapm.), the natural hybrid of longleaf (*Pinus palustris* Mill.) and loblolly pine (*Pinus taeda* L.), commonly occurs in longleaf pine seedlots grown in forest tree seedling nurseries in the southeastern United States. Because longleaf pine seedlings have a grass stage with minimal epicotyl development (< 1 cm), the initiation of stem growth (12 to 15 cm) in longleaf pine seedlots has been used to indicate that hybridization with loblolly pine (*Pinus taeda* L.) has occurred. Sonderegger pine seedlings are typically culled at the nursery due to observations of poor form and wood quality after outplanting. However, research documenting Sonderegger pine seedling morphology has not been published for more than 60 years, and to our knowledge, no seedling quality assessments have been made. To better understand how morphological traits of longleaf and loblolly pine are expressed in hybrid seedlings, stem length, hypocotyl length, and root collar diameter (RCD) were compared among one-year-old container-grown longleaf, loblolly, and seedlings visually classified as Sonderegger pine. Sonderegger pine seedlings had a range of stem development, with most (62%) seedlings measuring < 12 cm tall. Some pure longleaf pine seedlings had up to 10 cm of stem elongation, but the cause of early height growth in these seedlings is unknown. More than 90% of Sonderegger pine seedlings met or exceeded RCD recommendations for planting loblolly (≥ 3.2 mm) and longleaf pine (≥ 4.75 mm).

Keywords

Sonderegger pine; Longleaf pine; Loblolly pine; Morphology; Seedling quality

Contents

1 Introduction

Longleaf pine (*Pinus palustris* Mill.) forests once dominated the Southeastern Coastal Plain of the United States, occupying more than 36 million hectares (ha) from east Texas to southern Virginia prior to European settlement (Jose et al. 2006). However, extensive exploitation and land use conversion during the 19th and 20th centuries reduced the ecosystem to < 5% of its original range (Frost 2006). Much of the harvested area was abandoned to grassland, converted to agriculture, or planted with more economically attractive southern pines (Barnett and McGilvray 1997), and few seed trees remained to naturally regenerate cutover lands (Barnett 2002).

Of all southern pines, many consider longleaf pine most valuable in terms of wood-product quality, aesthetics, and tolerance to fire, insects, and diseases (Barnett 2002). Longleaf pine savannas and woodlands are among the most diverse ecosystems in temperate North America, having high levels of species richness and large numbers of endemic flora and fauna (Jose et al. 2006; Mitchell et al. 2006). The highest species richness is found in the herbaceous understory, where more than 1,000 plant species can be found over a few thousand hectares (Mitchell et al. 2006). Nearly two-thirds of all species that are recognized as declining, threatened, or endangered in the southeastern U.S. are associated with the longleaf pine ecosystem (Kirkman and Mitchell 2006).

Regenerating longleaf pine, whether naturally or artificially, is often considered more difficult than for any other southern pine species due to a period of delayed stem growth known as the "grass stage" (Barnett 2002; Brockway et al. 2006). The grass stage in longleaf pine is recognized as a fire-adapted trait that allows seedlings to tolerate and survive repetitive fires through needle insulation of the terminal bud (Aubrey 2021). During the grass stage, seedlings preferentially allocate assimilated carbon to taproot development and carbohydrate storage reserves, which are believed to re-foliate seedlings after prescribed burns (Aubrey 2021) and facilitate grass stage emergence (Brockway et al. 2006). Depending on vegetative competition at the planting site and frequency of prescribed burns, seedlings can remain in the grass stage from two to 15 years or longer (Wahlenberg 1946; Stambaugh et al. 2018). Regardless of seedling age, however, rapid height growth does not usually begin until seedlings have reached 2.5 cm in ground-line diameter and 10 cm in height (Wahlenberg 1946).

Increased interest in restoring longleaf pine to many sites in the southern United States has led to a significant rise in the demand for longleaf pine seedlings over the past few decades (Barnett and McGilvray 2002; Sung et al. 2013; Aubrey 2021). Planting container stock is recognized as the most successful method for regenerating longleaf pine (Barnett and McGilvray 1997). Numerous studies have shown that container-grown seedlings survive and grow better than bareroot seedlings under adverse planting conditions, such as poor sites, drought stress, and out-of-season plantings (South and Barnett 1986; Boyer 1989; Barnett and McGilvray 1993). Improved survival and growth of container seedlings is often attributed to root systems being protected in a plug of organic substrate, which helps prevent seedling injury at lifting and provides moisture and nutrient retainment for better field establishment (Barnett 2002).

Longleaf pine seeds sown in forest tree seedling nurseries in the southern United States are often collected from various sources (Barnett and McGilvray 2002; Weick et al. 2017; Olatinwo et al. 2020). In 2012, 73% of longleaf pine seedlots were collected from national forest lands that are designated as "seed production areas," while only 27% were collected from established seed orchards and considered "improved" genetic quality (Starkey et al. 2015). While most longleaf pine seeds collected from seed orchards are genetically pure (Olatinwo et al. 2020), a small percentage germinate and emerge in the nursery as the loblolly (*Pinus taeda* L.) and longleaf pine hybrid known as Sonderegger pine (*Pinus x sondereggeri* Chapm.) (Chapman 1922). Wahlenberg (1946) reported that 0.5 to 6% of a longleaf pine seedlot may contain hybrid seed, while Zobel and Talbert (1984) reported a 5 to 15% rate based on seedling production in large nurseries. Generally, a hybridization rate of < 3% in any longleaf pine seedlot is considered acceptable (Olatinwo et al. 2020).

Sonderegger pine has been considered an unmerchantable tree of poor wood quality for more than 100 years (van Buijtenen 1969; Dorman 1976). Wakeley considered the hybrid less desirable than either longleaf or loblolly pine due to susceptibility to numerous pests and large branches (pers. comm. to Schoenike et al. 1975). Most landowners desiring longleaf pine decline outplanting the hybrid seedlings to help ensure the genetic purity of forest stands and future seed crops (Guldin et al. 2016; Barnett et al. 2020; Jackson et al. 2020). For these reasons, Sonderegger pine seedlings are discarded during seedling extraction and processing at the nursery.

Currently, detecting Sonderegger pine seeds within a longleaf pine seedlot at the time of sowing is not possible without DNA analysis (Bolner et al. 2019; Olatinwo et al. 2020). Due to the grass stage growth habit of longleaf pine, the initiation of height growth (12 to 15 cm) has been used to distinguish Sonderegger pine from pure longleaf pine seedlings in the nursery (Chapman 1922; Wahlenberg 1946; Schoenike et al. 1975; Wilhite 1976; Schmidtling 1999; Hainds and Barnett 2004; Barnett et al. 2020). However, recent studies show that some pure longleaf pine seedlings may initiate height growth (up to 10 cm) (Hainds and Barnett 2006; Dumroese et al. 2009), causing them to be misidentified and discarded from the nursery as Sonderegger pine (Barnett et al. 2020; Jackson et al. 2020; Olatinwo et al. 2020). To our knowledge, no research has explained the cause of height growth in these longleaf pine seedlings, or whether it is caused by epicotyl development (Hainds and Barnett 2006; Barnett et al. 2020) or hypocotyl extension after germination (Bolner et al. 2019). Furthermore, limited information has been published on Sonderegger pine seedling morphology and development, especially for container-grown seedlings (Barnett et al. 2020).

Morphological attributes such as seedling height and root collar diameter (RCD) are often used to determine seedling quality in the nursery, and when combined with physiological attributes (e.g., root growth potential, nutrient status), help indicate the potential for seedling survival and field performance after outplanting. The earliest morphological grading standards were established for one-year-old bareroot pine seedlings based on stem diameter (RCD), stem height, presence of fascicle (secondary) needles, stem sturdiness, terminal bud condition, and presence of bark on the stem (Wakeley 1954). Seedlings are not typically sold according to morphological grade (South and Mexal 1984), but growth parameters initially suggested by Wakeley (1954) defined the long-standing relationship between seedling quality and outplanting performance. Numerous studies have shown that greater RCDs relate to better survival (South and Mexal 1984; Hainds and Barnett 2004, 2006; South et al. 2005) and help facilitate grass stage emergence in longleaf pine seedlings after outplanting (Wahlenberg 1946; Jackson et al. 2012). Morphological parameters of seedling quality are well defined for loblolly (Wakeley 1954; Brissette and Lantz 1983; May 1984) and longleaf pine (Barnett et al. 2002; Hainds and Barnett 2004, 2006; Dumroese et al. 2009), but morphological traits exhibited by Sonderegger pine and their relationship to field performance are unknown. The objectives of this research were to (1) compare stem length, hypocotyl length, and RCD of container-grown Sonderegger, longleaf, and loblolly pine seedlings and (2) provide Sonderegger pine seedling data that may be used to develop seedling quality standards that have similarly been defined for longleaf and loblolly pine.

2 Materials and methods

2.1 Seedling acquisition

On January 25, 2023, one-year-old container-grown loblolly pine, longleaf pine, and seedlings selected as Sonderegger pine were acquired from the PRT-IFCO nursery in Moultrie, GA (31° 13' 43" N, 83° 48' 23" W). Seedlings had been grown in the nursery using FT128-Forestry Trays (Stuewe and Sons, Inc., Tangent, OR), and each tray had 128 cavities (560 cavities m^{-2}) with individual volumes of 131 cm^3. Nursery personnel selected loblolly pine seedlings from two controlled mass-pollinated families (CMP 04922, CMP 05122) and longleaf pine seedlings from two improved open-pollinated families (IMP 008, IMP 011). Seedlings that exhibited stem elongation in the same longleaf pine families (IMP 008, IMP 011) were also collected and classified as Sonderegger pine. All seedlings were placed in cold storage at Louisiana Tech University on January 25, 2023 to allow measurements to be conducted over the next two months.

Upon sorting through the seedlings in the laboratory at Louisiana Tech, some seedlings selected as Sonderegger pine had up to 10 cm of stem elongation but had bud characteristics indicative of pure longleaf pine. Some of these elongated seedlings had a large sessile bud typical of an overwintering longleaf pine, while other seedlings lacked buds and secondary needle development which is often seen in weak, low quality longleaf pine seedlings (Wakeley 1954; Jackson 2006). Because the seedlings selected as Sonderegger pines in this trial were showing stem elongation above and below 10 cm, seedlings were placed into two phenotypic categories for morphological assessment according to bud characteristics only. Seedlings that were selected as Sonderegger pine but had bud characteristics indicative of pure longleaf pine were labeled as "Sonderegger pine with longleaf traits" (hereafter "SP-LT" seedlings) (Figure 1a). Seedlings with an elongated reddish-brown bud were classified as Sonderegger pine (Figure 1b).

Figure 1. Classification of SP-LT and Sonderegger pine seedlings. (left) This seedling was operationally identified as a Sonderegger pine but classified in the laboratory as an SP-LT seedling due to its sessile bud, which is typical of longleaf pine seedlings in the grass stage. (right) This seedling was operationally identified as a Sonderegger pine and classified as a hybrid in the study. The seedling measured 14.4 cm tall and had intermediate bud characteristics between longleaf and loblolly pine.

2.2 Nursery seedling morphology

2.2.1 Stem length and diameter

From January 30 to March 31, 2023, measurements of stem length, hypocotyl length, and RCD were recorded on 199 longleaf pine, 100 loblolly pine, 203 Sonderegger pine, and 49 SP-LT seedlings (551 seedlings total). Stem length was measured from the cotyledon scar to the tip of the terminal bud (Brown 1964; Mexal and Landis 1990). When no obvious terminal bud was present, stem length was taken from the swollen part of the shoot tip indicating the location of the terminal meristem (Mexal and Landis 1990). Hypocotyl length was measured from the cotyledon scar to the first divergence of a lateral root (Bolner et al. 2019), and RCD was taken 1 cm above the first order lateral root (Mexal and Landis 1990).

2.2.2 Bud development on longleaf pine and SP-LT seedlings

To describe bud morphology, a classification system was modified from the collective works of Pessin (1939), Wahlenberg (1946), Wakeley (1954), and Brown (1964). The number of buds were counted on each longleaf pine and SP-LT seedling and categorized into one of five development stages based on the morphology of the dominant terminal bud: (1) resting, (2) round, (3) pincushion, (4) needle elongation, or (5) no bud (Table 1; Figure 2). For Sonderegger pine seedlings, the number of buds were counted, and the height and diameter of the dominant terminal bud was measured.

Table 1. Bud development stages used to describe one-year-old longleaf pine and SP-LT seedlings.

Description	Bud Stage	Any green color present	Classification
Bud is tightly scaled.	Resting	No	1A
		Yes	1B
Bud is loosely scaled and slightly convex.	Round	No	2A
		Yes	2B
Bud scales are fully open, exposing unopened needle sheaths; few or no discernible bud scales remain. Bud surface is hard and flat or slightly convex.	Pincushion	N/A	3
Less than 50% of needles have elongated from the bud set area.	Needle elongation	N/A	4
More than 50% of needles have elongated from the bud set area; no hard surface remains.	No bud	N/A	5

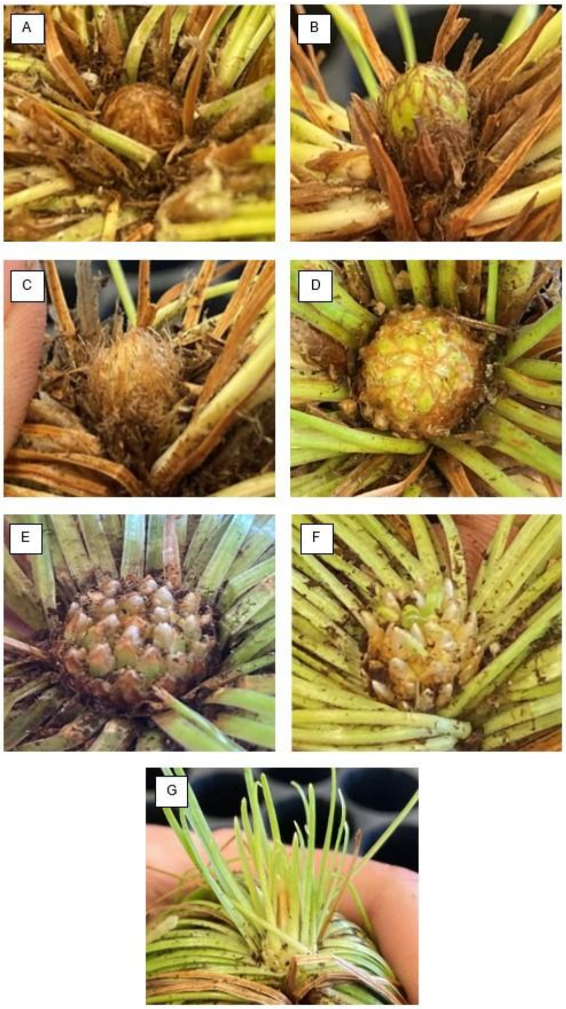

Figure 2. Bud development stages used to categorize longleaf pine and SP-LT seedlings. (A) Stage 1A-resting bud with no green present; (B) Stage 1B-resting bud with green present; (C) Stage 2A-round bud with no green present; (D) Stage 2B-round bud with green present; (E) Stage 3-pincushion; (F) Stage 4-needle elongation; (G) Stage 5-no bud.

2.2.3 Data analysis

To compare stem length, hypocotyl length, and RCD among seedling types, an analysis of variance (ANOVA) was conducted using a General Linear Model, and multiple comparisons of means were conducted using Duncan's Multiple Range Test (9th ed., SAS Institute, Cary, NC). Relationships among designated seedling types and the dependent variables were assessed with Principal Component Analysis (PCA). This was implemented in R-software and data were plotted across the first two components which accounted for 93% of all variance. Data points were colored by seedling type to better determine clustering of species by traits. A chi-square test was used to determine if bud development stages in SP-LT and longleaf pine seedlings occurred with equal probability.

2.3 DNA analysis and transplanted seedling morphology

2.3.1 Growth and morphology

To further conduct species comparisons and determine if SP-LT seedlings were hybrids or pure longleaf pines, needle samples were collected from longleaf pine (n = 10), Sonderegger pine (n = 10), and SP-LT seedlings (n = 9) and analyzed for paternal loblolly pine chloroplast DNA (Olatinwo et al. 2020). On March 13, 2023, the 29 seedlings that were sampled for DNA analysis and an additional 10 loblolly pine seedlings were transplanted into black one-gallon nursery containers (Classic® 400, Nursery Supplies, Inc., Chambersburg, PA) to monitor their growth and development. Containers were filled with a peat-moss based substrate with a pH of 6.0 (LSU Soil Testing and Plant Analysis Lab, Baton Rouge, LA) at the time of transplanting. Containers were randomized and placed outside in full sun on growing benches at Louisiana Tech University (32° 31' 02" N, 92° 39' 08" W).

From April 14 to June 1, 2023, seedlings were liquid-fertilized with 1 L of 30-10-10 Miracle Gro® (Marysville, OH) solution every 14 to 21 days using the recommended rate of 3.9 mL L^{-1}. From June 8 to August 30, applications of liquid fertilizer continued every 14 to 21 days at double the recommended rate (7.8 mL L^{-1}) to accommodate rapid seedling growth. In addition, on May 5 and June 1, 15 g of 14-14-14 Osmocote® slow-release fertilizer (Marysville, OH) was applied as a top-dressing to each seedling.

Seedling height and stem diameter were recorded at transplanting (week 0) and 12 and 24 weeks after transplanting. Height was measured from the top of the media surface to the tip of the terminal bud (Mexal and Landis 1990). When no terminal bud was present, height was taken from the swollen part of the shoot tip indicating the location of the terminal meristem (Mexal and Landis 1990). Stem diameter was measured at the substrate surface (Mexal and Landis 1990). Bud counts and development stages (Table 1; Figure 2) were documented for all Sonderegger pine, longleaf pine, and SP-LT seedlings at 24 weeks.

2.3.2 Data analysis

Repeated measures ANOVAs (SPSS, version 26) were used to examine differences in height and RCD among seedling types throughout the 24-week period, and Tukey *post hoc* tests were used to analyze differences among types. Because the sphericity assumption was violated for both tests, we report the adjusted degrees of freedom and P-values based on the Huynh-Feldt correction (Haverkamp and Beauducel

2017), which corrects for the sphericity violation and is more efficient and has greater statistical power than the Greenhouse-Geiser correction (Abdi 2010).

3 Results

3.1 Nursery seedling morphology

3.1.1 Stem length and diameter

Stem length of loblolly, longleaf, Sonderegger pine, and SP-LT seedlings were significantly different (P < 0.001) (Table 2). Loblolly pine seedlings were 18.5 cm taller than Sonderegger pine seedlings with a mean stem length of 29.9 cm and 11.4 cm, respectively. Sonderegger pine seedlings were 9.5 cm taller than longleaf pine seedlings and 6.6 cm taller than SP-LT seedlings. Mean stem length of SP-LT seedlings was 4.8 cm, which indicated intermediate growth between Sonderegger and longleaf pine seedlings. Stem length of Sonderegger pine seedlings was the most variable, ranging from 3.7 to 26.1 cm. Of the 203 Sonderegger pine seedlings measured, 125 (62%) had stem lengths < 12.0 cm.

Loblolly pine seedlings had the longest hypocotyls at 3.6 cm, and longleaf pine hypocotyls were the shortest at 1.4 cm (P < 0.001) (Table 2). Hypocotyl lengths of SP-LT and longleaf pine seedlings were not significantly different. Hypocotyl length of Sonderegger pine seedlings was 0.7 cm shorter than loblolly pine and 1.5 cm longer than both longleaf pine and SP-LT seedlings. There was a significant difference in RCD among all seedling types (P < 0.001) (Table 2). Loblolly pine had the smallest mean RCD ranging from 3.0 to 6.6 mm, while longleaf pine had the largest mean RCD ranging from 5.0 to 12.9 mm. On average, longleaf pine seedlings were 0.9 and 2.2 mm larger in diameter than SP-LT and Sonderegger pine seedlings, respectively.

Table 2. Bud development stages used to describe one-year-old longleaf pine and SP-LT seedlings.

Pine	Stem Length (cm)	Hypocotyl Length (cm)	RCD (mm)
Loblolly	29.9 a	3.6 a	4.5 d
Sonderegger	11.4 b	2.9 b	6.1 c
SP-LT	4.8 c	1.5 c	7.4 b
Longleaf	1.9 d	1.4 c	8.3 a
P-value	< 0.001	< 0.001	< 0.001

The cluster analysis suggested two distinct species groups, an intermediate hybrid group, and an overlapping group of SP-LT seedlings (Figure 3). The first principal component accounted for 81% of the variation of the three variables measured (i.e., stem length, hypocotyl length, and RCD) for all samples. PC1 had a positive correlation with RCD with a loading of 0.55 and negative correlations with hypocotyl length and stem length, which were both -0.58. PC2 had positive loadings with all variables, with RCD, stem length, and hypocotyl length having loadings of 0.82, 0.39, and 0.40, respectively. The clustering of seedling types was evident across the PC1 component which captured the inverse relationship between seedling height and RCD. Longleaf pine and SP-LT seedlings tended to have higher values of PC1, while loblolly pine seedlings had lower values of PC1. Most (86%) SP-LT seedlings were grouped within the defined longleaf pine cluster, while the remaining seedlings overlapped the

Sonderegger pine cluster (6%) or were exclusively grouped in the SP-LT (4%) or Sonderegger pine clusters (4%). Only one Sonderegger pine seedling was grouped in the longleaf pine cluster. Loblolly pine was clearly distinguished from all other seedling types using PC1 alone.

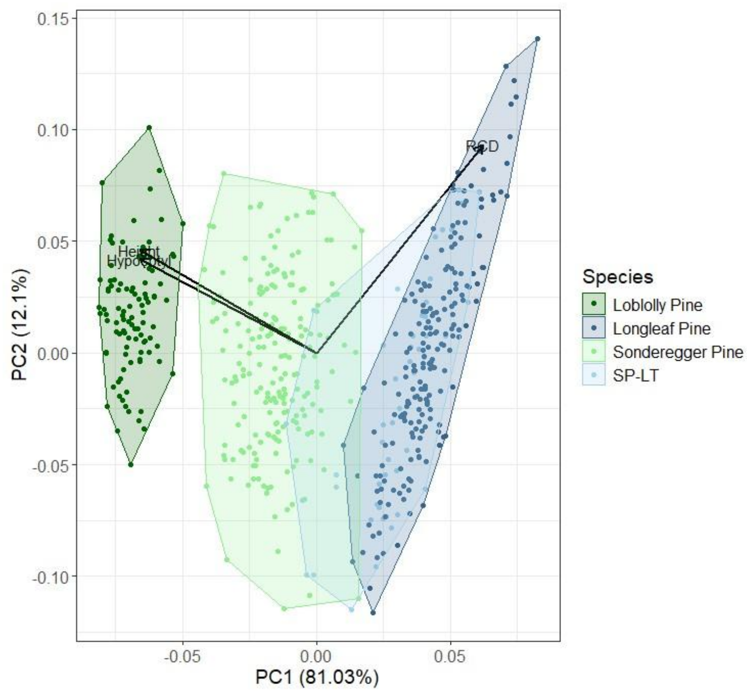

Figure 3. PCA loadings of stem length (height), hypocotyl length, and RCD in loblolly pine, longleaf pine, Sonderegger pine, and SP-LT seedlings.

3.1.2 Bud development

Resting buds of Sonderegger pine were covered in reddish-brown scales (resembling loblolly pine) and sharply pointed at the apex (resembling vigorous longleaf pine seedlings). On average, Sonderegger pine seedlings had one terminal bud, but some seedlings had up to five. The mean length and width of resting buds was 19 mm and 5 mm, respectively. However, the bud in one seedling developed atypically with a length of 148 mm, comprising nearly 60% of total stem length. This elongated bud was not woody like a true stem, but flesh-like, tapered at the apex, and covered in reddish-brown bud scales.

All longleaf pine seedlings (n = 199) and 94% of SP-LT seedlings (n = 46) had a single discernible bud that showed a wide range of developmental stages ($\chi^2_{4,245} = 45.3$; $P < 0.001$) (Figure 4). More than 70% of longleaf pine seedlings had a round bud that was loosely scaled and preparing to open. The remaining longleaf pine seedlings had buds either in the resting (16%), pincushion (9%), or needle elongation (3%) stages. Nearly 60% of SP-LT seedlings were in the resting or round bud stage. The remaining SP-LT seedlings had buds in the pincushion (16%), needle elongation (22%) or no bud (6%) stage. Compared to longleaf pine, SP-LT seedlings had reached more mature stages of development (i.e., 30% more seedlings in the pincushion or needle elongation stage).

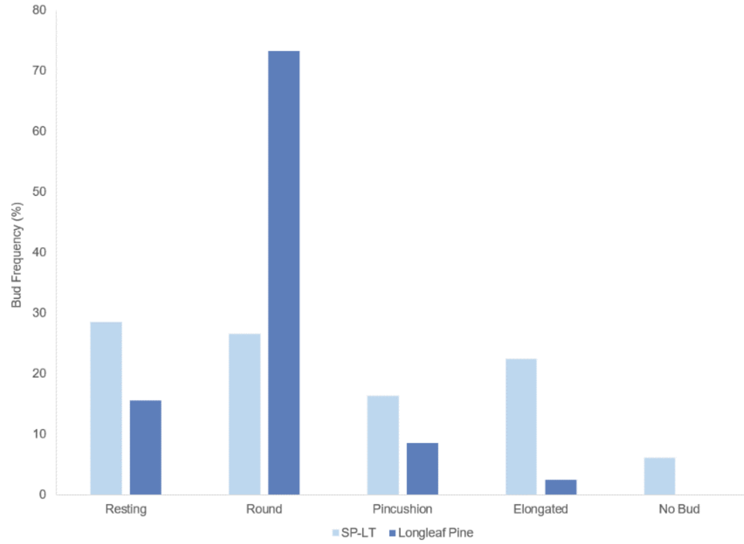

Figure 4. Frequency of bud stages observed in longleaf pine and SP-LT seedlings acquired from the PRT-IFCO nursery in Moultrie, GA in January 2023.

3.2 DNA analysis and transplanted seedling morphology

3.2.1 DNA analysis

All 10 Sonderegger pine seedlings sampled for DNA analysis tested positive for loblolly pine paternal chloroplast DNA, indicating that the seedlings were hybrids. All longleaf pine (n = 10) and SP-LT seedlings (n = 9) tested negative for loblolly pine DNA, indicating that they seedlings were pure longleaf pines.

3.2.2 Transplanted seedling morphology

At transplanting, loblolly pine seedlings were tallest followed by Sonderegger pine, SP-LT, and longleaf pine seedlings (Figure 5). This pattern in height growth continued throughout the 24-week period after transplanting ($F_{4,31} = 53.52$; $P < 0.001$). Loblolly pine seedlings grew faster during the first 12 weeks compared to any other seedling type with height growth of about 18 cm, followed by Sonderegger pine at nearly 8 cm. Loblolly and Sonderegger pine seedlings grew at similar rates during the last 12 weeks of the study with mean height growth of about 14 cm. Both longleaf pine and SP-LT seedlings grew less than 5 cm during the 24-week period ($P = 0.119$).

At the time of transplanting, longleaf pine had the largest stem diameter at 10.3 mm, followed by SP-LT seedlings, Sonderegger pine, and loblolly pine (Figure 6). After 24 weeks, Sonderegger pine had the largest stem diameter at 18.7 mm, which was 1.7 mm larger on average compared to longleaf pine, SP-LT seedlings, and loblolly pine ($F_{4,31} = 6.21$; $P < 0.001$). Both Sonderegger and loblolly pine grew at a faster rate compared to longleaf pine and SP-LT seedlings with an increase of approximately 12 mm in stem diameter during the 24-week period. During the first 12 weeks, loblolly pine gained almost 4 mm in diameter, followed by Sonderegger pine (3 mm), SP-LT seedlings (2.5 mm), and longleaf pine (< 1 mm). From weeks 12 to 24, Sonderegger pine grew the fastest with 8.8 mm, followed by loblolly pine (8.3 mm), SP-LT seedlings (7.1 mm), and longleaf pine (6.3 mm).

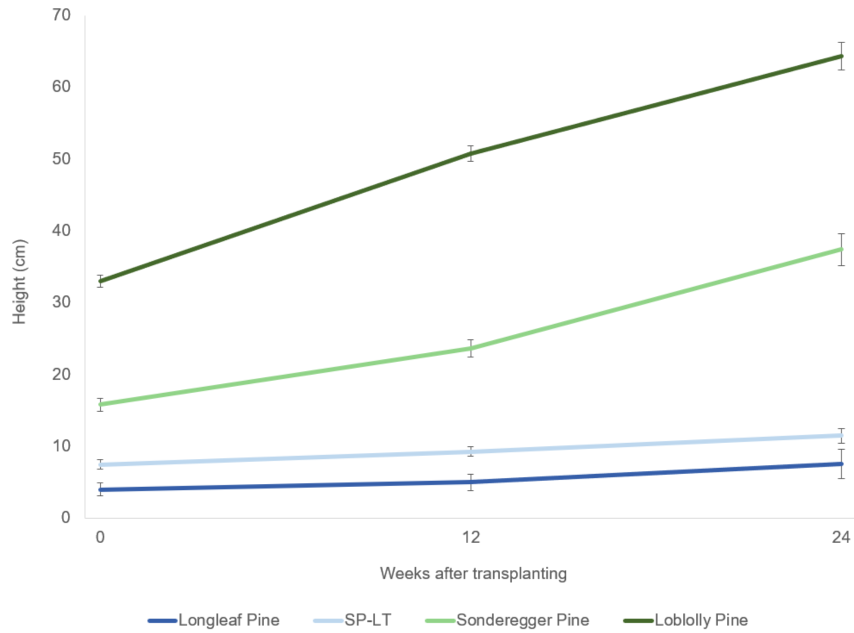

Figure 5. Mean height of loblolly pine, Sonderegger pine, longleaf pine, and SP-LT seedlings ($F_{4,31}$ = 53.52; P < 0.001) at 0, 12, and 24 weeks after transplanting into one-gallon nursery containers in the 2023 growing season. Error bars represent ±SE of the mean.

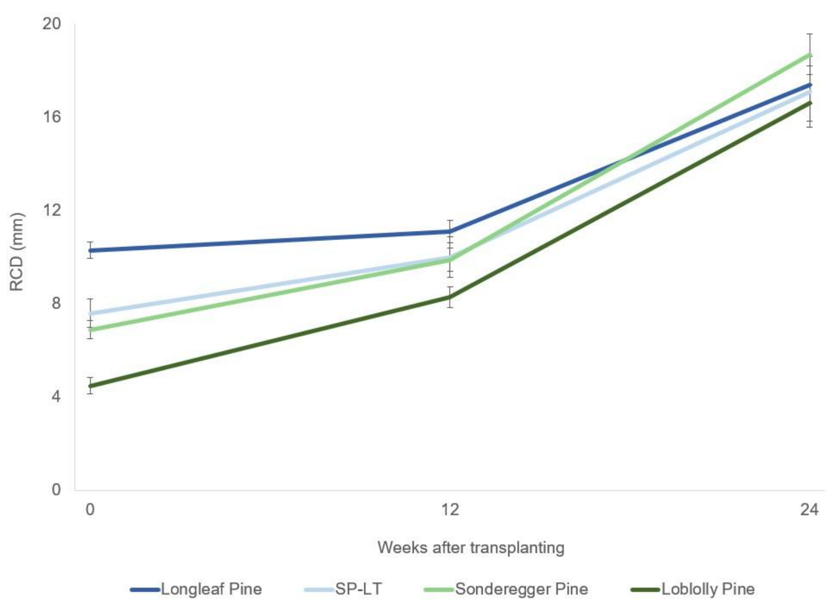

Figure 6. Mean RCD of loblolly pine, Sonderegger pine, longleaf pine, and SP-LT seedlings ($F_{4,31}$ = 6.21; P< 0.001; α = 0.05) at 0, 12, and 24 weeks after transplanting into one-gallon nursery containers in the 2023 growing season. Error bars represent ±SE of the mean.

Seedling types showed distinct morphologies 24 weeks after transplanting (Figure 7). Loblolly and longleaf pine seedlings developed as expected with loblolly pines developing multiple lateral branches and longleaf pines remaining in the grass stage. Some Sonderegger pine seedlings developed woody lateral branches that diverged from the main stem, while other seedlings lacked woody branch growth entirely. These seedlings had tufts of secondary needles that emerged from lateral nodes on the main stem, resembling longleaf pines that are bolting out of the grass stage. Stems and branches of Sonderegger pine seedlings were covered in secondary needles, but foliar density varied from seedling to seedling. All SP-LT seedlings remained in a grass stage similar to longleaf pine.

Figure 7. Left to right: Typical form observed in loblolly pine, Sonderegger pine, SP-LT, and longleaf pine seedlings 24 weeks after transplanting.

3.2.3 Bud development

Buds were present on two of the nine longleaf pine seedlings 24 weeks after transplanting. One bud was in the resting stage, and one bud was in the round stage. The resting longleaf pine bud was white, covered with brown scales, and sharply pointed at the apex (Figure 8A). All loblolly and Sonderegger pine seedlings had buds 24 weeks after transplanting. Loblolly pine buds in the resting stage were covered in reddish-brown scales, longer than wide, and pointed less sharply at the apex compared to longleaf pine seedlings (Figure 8). Sonderegger pine buds were intermediate between longleaf and loblolly pine in size, color, and apex features. Buds in the resting stage were white, covered in reddish-brown scales, and pointed more sharply at the apex compared to loblolly pine seedlings. On average, Sonderegger pine seedlings developed three buds, but the number of buds per seedling ranged from one to five. No SP-LT seedlings had a discernible bud at 24 weeks. However, it was apparent that buds were present previously because some needles were not fully elongated from the bud set area.

Figure 8. Typical resting buds of (A) longleaf pine, (B) Sonderegger pine, and (C) loblolly pine seedlings 24 weeks after transplanting.

4 Discussion

4.1 Nursery seedling morphology

About 25% of seedlings selected from the nursery as Sonderegger pines had bud characteristics indicative of longleaf pine and were classified in this study as SP-LT seedlings. Further morphological evaluations and DNA analysis revealed that the SP-LT seedlings were pure longleaf pine. For decades, stem growth (12 to 15 cm) observed among longleaf pine seedlings has been used to identify Sonderegger pine in the nursery (Barnett et al. 2020). In our study, stem length of Sonderegger pines ranged from 4 to 26 cm with most having stems < 12 cm (62%). Stem development in SP-LT seedlings ranged from 2 to 10 cm, demonstrating that stem elongation in longleaf pine seedlots may not be the best indicator of hybridization.

In describing longleaf pine germination, Boyer (1990) stated that “the epicotyl, or stem above the cotyledons, does not elongate rapidly as in most other pines. Even in the nursery, seedlings are virtually stemless after one growing season.” Similarly, Brockway et al. (2006) stated “although secondary needles appear within 2 months [after germination], the epicotyl reaches a length of only 0.38 cm and, during the next 8 weeks, does not elongate as in other pines.” In our study, longleaf pine seedlings with normal development measured 1.9 cm tall, while SP-LT seedlings averaged 4.8 cm. The cause of stem development in SP-LT seedlings remains unknown, but similar growth patterns have been documented in pure longleaf pine seedlings in recent years (Barnett and McGilvray 2006; Barnett et al. 2020). Longleaf pine seeds collected from a Louisiana and Mississippi source in 2014 produced seedlings that developed stem elongation that was considered abnormal. Because these seedlings were suspected as being Sonderegger pine, nursery managers chose not to process them for sale (Jackson et al. 2020). In 2016, field plots of these suspected hybrid seedlings were established for long-term observations (Jackson et al. 2020). Jackson et al. (2020) reported that the suspected hybrid seedlings remained in the grass stage two years after outplanting and tested negative for loblolly pine DNA (Olatinwo et al. 2020), indicating that they were pure longleaf pines.

Sherry (1947) was the first to demonstrate that longleaf pine seedlings can exhibit a range of stem elongation in the nursery. In 1941, Sherry (1947) established plots of one-year-old longleaf pine seedlings and suspected hybrids collected from the same longleaf pine seedlots for long-term observational study. At outplanting, the suspect hybrid seedlings ranged from 15 to 30 cm in height, while control seedlings were in the grass stage with stem lengths of about 2.5 cm. Four years after outplanting, nearly 70% of the suspected hybrids were ≥ 6 m tall and 10 cm in DBH, while control seedlings were < 6 m tall. Based on these observations and other morphological characteristics at age five, 30% of the suspected hybrids were determined to be longleaf pine.

The cause of stem elongation in some longleaf pine seedlings is currently unknown, but findings from this study suggest that this type of development may be more common than once realized. Brown (1964) reported that stem length and hypocotyl length were weakly correlated in F_1 and F_2 Sonderegger pine seedlings, concluding that two different genetic mechanisms control the growth and differentiation of the hypocotyl and epicotyl. More research is needed to determine the genetic and environmental interactions that influence stem development in longleaf and Sonderegger pine seedlings.

Maximum length of longleaf pine hypocotyls (~1 cm) occurs by the eighth day after the onset of germination (Brown 1964). In contrast, loblolly pine hypocotyls elongate up to 18 days after germination, reaching a final length of about 3.5 cm (Brown 1964; Schultz 1997). Brown (1964) reported that Sonderegger pine seedling hypocotyls exhibited intermediate growth, reaching maturity after 14 days at 2.3 cm. In our study, Sonderegger pine seedling hypocotyl length was intermediate between longleaf and loblolly pine at 2.9 cm.

In describing longleaf pine seedling development, Boyer (1990) stated that "newly germinated seedlings have virtually no aboveground hypocotyl, and the cotyledons are close to the ground line." Thus, in recent years, a theory was proposed that hypocotyl extension above the substrate surface (i.e., "hypocotyl lift") in some longleaf pine seedlings indicated that hybridization with loblolly pine had occurred. While testing this theory, Bolner et al. (2019) found that limited light availability and high media surface temperature as influenced by container color and sowing depth may contribute to hypocotyl lift in longleaf pine seedlings. Bolner et al. (2019) reported that longleaf pine seedlings grown in black containers had more hypocotyl lift compared to those grown in white containers, and seedlings grown in cavities filled to two-thirds capacity (12.7 cm) had more hypocotyl lift compared to cavities filled to operational levels (7.6 cm). However, total hypocotyl length (i.e., distance from the first order lateral root to the cotyledon scar) was not significantly different between treatments. Their research supports findings from Brown (1964), who stated that "the intensity of light and varying temperature conditions during the period of hypocotyl elongation has little effect on final hypocotyl length in longleaf pine." Collectively, these studies suggest that (1) hypocotyl lift is not a reliable indicator of hybridization and (2) final hypocotyl length in longleaf pine seedlings is under strong genetic control.

Bud evaluations conducted during the nursery phase indicated that all loblolly and Sonderegger pine seedlings had resting buds, while longleaf pine and SP-LT seedlings varied in stages of development ranging from resting buds to fully extended needles. Schultz (1997) described resting loblolly pine buds as reddish brown, conical shaped, and longer than wide, averaging 10 to 15 mm long and 4 to 6 mm wide. In our study, Sonderegger pine buds in the resting stage were slightly larger, averaging 19 mm long and 5 mm wide, and more sharply pointed at the apex compared to loblolly pine. Sonderegger pine resting buds were covered in brown to reddish-brown scales, later revealing sharply pointed, vigorous white buds after their second growing season (24 weeks after transplanting). This may be the first report of Sonderegger pine bud morphology at any growth stage. Because only resting buds of loblolly and Sonderegger pine were able to be compared, further research is needed to document Sonderegger pine bud morphology throughout various stages of seedling development.

4.2 Sonderegger pine seedling quality

Longleaf pine RCDs at outplanting should be ≥ 4.75 mm, but larger RCDs (i.e., ≥ 6.35 mm) are recommended (Wakeley 1954; Dumroese et al. 2009). Seedlings below the 4.75 mm threshold may have poor survival after outplanting (Wakeley 1954; Hainds and Barnett 2004, 2006), and larger RCDs are correlated to reduced time in the grass stage (Jackson et al. 2012). Generally, most fertilization regimes in container nurseries produce longleaf pine seedlings above the minimum RCD threshold (Jackson 2006; Dumroese et al. 2009). In our study, 98% of longleaf pine and SP-LT seedlings had RCDs ≥ 4.75 mm. Approximately 90% of all seedlings classified as Sonderegger pine measured ≥ 4.75 mm in diameter, with 38% measuring ≥ 6.35 mm.

Root collar diameter of loblolly pine seedlings should be ≥ 3.2 mm at outplanting (Wakeley 1954; Brissette and Lantz 1983; Venator 1983; May 1984), but RCDs of 5.6 to 9.5 mm are recommended (Venator 1983; May 1984). Of the loblolly pine seedlings measured, 98% had RCDs ≥ 3.2 mm, but only 7% had RCDs ≥ 5.6 mm. All seedlings classified as Sonderegger pine measured above the 3.2 mm threshold, with 72% measuring ≥ 5.6 mm.

Ideal seedling height in loblolly pine varies depending on soil type, moisture stress, and competitive vegetation at the outplanting site (South and Mexal 1984). Because seedling height is correlated to the number of needles on a seedling, it also provides an estimate of photosynthetic potential and transpirational area after outplanting (Haase 2008). On sites with vegetative competition, taller seedlings may outperform shorter seedlings, but on droughty sites, shorter seedlings with lower transpirational area often have better survival and growth after outplanting (May 1984; South and Mexal 1984). Brissette and Lantz (1983) recommend that loblolly pine seedlings should be at least 18 cm tall at outplanting, but South and Mexal (1984) found that seedlings ranging from 13 to 31 cm had similar survival and growth one year after outplanting when soil moisture was not a limiting factor. In our study, stem length of Sonderegger and loblolly pine seedlings ranged from 4 to 26 cm and 23 to 36 cm, respectively. To our knowledge, no studies have examined how seedling height at outplanting correlates to Sonderegger pine field performance across various site conditions.

4.3 Transplanted seedling morphology

The grass stage in longleaf pine seedlings is a defining characteristic of the species (Wahlenberg 1946; Boyer 1990; Brockway et al. 2006; Aubrey 2021). According to Wahlenberg (1946), grass stage emergence occurs when stem height reaches 10 cm and diameter at the ground line is 25 mm. In our study, SP-LT seedlings remained in the grass stage after their second growing season (24 weeks after transplanting), averaging 12 cm tall and 17 mm in ground-line diameter. These findings are consistent with previous observations indicating that early stem development in longleaf pine seedlings may not necessarily confer rapid grass stage emergence after outplanting (Sherry 1947; Jackson et al. 2020). Further research is needed to determine the mechanisms that control early stem development in longleaf pine seedlings and how they respond to silvicultural practices after outplanting.

5 Conclusion

Findings from this study reveal that pure longleaf pine seedlings can initiate up to 10 cm of stem growth, which may cause them to be misidentified and culled in the nursery as Sonderegger pine (i.e., SP-LT seedlings). We therefore conclude that when used alone, the initiation of stem growth in longleaf pine nursery stock is not the best indicator of hybridization. Until a more reliable indicator can be identified, bud characteristics may be used to help distinguish hybrids from pure longleaf pine nursery stock.

The cause of early stem development in some longleaf pine seedlings during nursery production is currently unknown, and it is uncertain how outplanting performance of these seedlings compares with normally developed longleaf pine seedlings. Our research raises several questions, including: (1) Is stem elongation in some longleaf pine seedlings facilitated by genetics, fertility, and/or the microenvironment in container cavities? (2) Could longleaf pine seedlings with early height growth exit the grass stage more quickly than seedlings with normal epicotyl development? (3) Do longleaf pine seedlings with early height growth have buds that are vulnerable to flames, potentially resulting in mortality from prescribed burns?

When compared with longleaf and loblolly pine, Sonderegger pine seedlings were intermediate in stem length, hypocotyl length, and RCD. Based on seedling quality standards, more than 90% of Sonderegger pine seedlings met RCD recommendations for either loblolly or longleaf pine seedlings. Future studies that evaluate physiological attributes (e.g., drought tolerance, root-growth potential) of Sonderegger pine seedlings would provide further insight into seedling quality and potential outplanting performance.

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