

Linking seed and leaf litter traits to post-fire recovery and forest flammability in southeastern U.S. forests

by

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Abstract

Decades of fire exclusion affected upland forests of the southeastern United States in many ways, including reductions in successful natural regeneration of fire-dependent species and shifts in forest flammability. To better understand natural regeneration of southern pine species, we tested the seed germination responses of southern pine species to increased soil temperature and decreased soil moisture and investigated relationships between cone production and seed production, size, and germination in longleaf pine. Because forest flammability is heavily driven by species composition and leaf litter fuel traits, but also top-down controls associated with climate/weather and fire history, we integrated conventional measurements of leaf litter traits associated with flammability at the species level across regional/climatic and fire frequency gradients. Recognizing nuances of seed and leaf litter traits will provide natural resource managers with better understanding of post-fire recovery and forest flammability in these forest systems.

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Table of Contents

Abstract.....	2
Acknowledgments.....	3
Table of Contents.....	5
List of Tables	7
List of Figures.....	8
List of Abbreviations	11
Chapter 1. How do climate and life-history impact regeneration success of southern pine species?	12
Abstract.....	12
Introduction.....	12
Methods.....	18
Soil Temperature and Soil Moisture Experiment	18
Study Sites for Longleaf Pine Seed Collection.....	21
Longleaf Pine Seed Collection.....	22
Longleaf Pine Seed Size	25
Collected Longleaf Pine Seeds Germination Trial	26
Data Analysis	26
Results.....	28
Soil Temperature and Soil Moisture Experiment	28
Longleaf Pine Seed Collection Results.....	30
Longleaf Pine Seed Size Results.....	33
Collected Longleaf Pine Seeds Germination Trial Results.....	34

Discussion	34
Soil Temperature and Soil Moisture Experiment	35
Longleaf Pine Seed Collection.....	39
Longleaf Pine Seed Size	40
Collected Longleaf Pine Seeds Germination Trial	41
Conclusions.....	42
Chapter 2. Comparison of leaf litter traits across species, regional/climatic, and burn frequency gradients in the southeastern United States	43
Abstract.....	43
Introduction.....	43
Methods.....	49
Study Areas.....	49
Litter Collection	52
Litter Processing	54
Data Analysis	55
Results.....	55
Discussion.....	62
Conclusions.....	65
References.....	67

List of Tables

Table 1. Comparison of linear model and quadratic model for total seeds, developed seeds, and undeveloped seeds collected/m ² versus cone count (x).	31
Table 2. Mean (SE) seed size measurements for longleaf pine seeds collected from Blackwater River State Forest (2022), Eglin Air Force Base (2022), and the Jones Center at Ichauway (2021 and 2022).	33
Table 3. Litter collection site information, including location, annual precipitation (cm), annual average temperature (°C), forest type, and prescribed fire frequency. All sites are in the U.S.A. Climate information from the National Oceanic and Atmospheric Administration National Centers for Environmental Information’s U.S. Climate Normals data.	51
Table 4. Species of litter sampled from each site. An asterisk (*) means that the species was collected at the site, but it was not collected at all fire frequency levels there. Z means that sand post oak (<i>Quercus margarettae</i> Small) was collected at one site at the Jones Center where post oak was not available, and Florida maple (<i>Acer floridanum</i> hort.) was collected at the Jones Center where red maple was not available.	53
Table 5. Principle component loadings for measured leaf litter traits in individual leaf data principal components analysis.	56

List of Figures

Figure 1. Experimental design for greenhouse germination trial. Heating mats for increased temperature treatments are represented by red rectangles. Trays in each moisture treatment are divided by the black line down the center of the diagram. The species treatments, shown by colors in each cell, are as follows: longleaf pine (*Pinus palustris* Mill.), shortleaf pine (*P. echinate* Mill.), loblolly pine (*P. taeda* L.), slash pine (*P. elliottii* Engelm.), and no seed (for temperature and moisture monitoring). The experiment occurred on one bench in the greenhouse, with the top of the figure representing the end of the bench closest to the window and the bottom of the figure representing the end of the bench closest to the center of the greenhouse. 20

Figure 2. Longleaf pine seed collection sites, including Blackwater River State Forest (Milton, Florida, U.S.A.), Eglin Air Force Base (Fort Walton Beach, Florida, U.S.A.), and the Jones Center at Ichauway (Newton, Georgia, U.S.A.). 22

Figure 3. Examples of 0.25-m² seed traps from the 2021 longleaf pine seed collection and 3-m² seed traps from the 2022 longleaf pine seed collection. 24

Figure 4. Examples of seeds from the different categories of collected seeds. Developed seeds are fully developed and fit for use in a germination trial. Undeveloped seeds are wings that did not show signs of developing into a viable seed. Damaged seeds are developed seeds that were damaged through attempted predation or other means..... 25

Figure 5. Percent germination (\pm SE) for loblolly, longleaf, shortleaf, and slash pine seeds on the final day of the greenhouse germination trial. 29

Figure 6. Percent germination over time of loblolly, longleaf, shortleaf, and slash pine seeds throughout the 60-day greenhouse germination trial. 30

Figure 7. Total number of seeds collected/m², developed seeds collected/m², and undeveloped seeds collected/m² versus number of cones produced on each tree at Blackwater River State Forest (2022), Eglin Air Force Base (2022), and the Jones Center at Ichauway (2021 and 2022). 32

Figure 8. Germination by seed mass (g) of longleaf pine seeds (black points) and predicted germination (blue line) for a given seed mass based on a logistic model of the germination trial results. 33

Figure 9. Percent germination of longleaf pine seeds collected from Blackwater River State Forest (2022), Eglin Air Force Base (2022), and the Jones Center at Ichauway (2021 and 2022) versus number of cones produced per tree. 34

Figure 10. Map of the twelve leaf litter collection locations. 50

Figure 11. Correlation plot for measured leaf litter traits. Darker blue with larger circles represents stronger positive correlation, and darker red with larger circles represents stronger negative correlation between two traits. 56

Figure 12. Principal components analysis plot using individual leaf litter trait data, grouped by species. Each point represents an individual leaf's measurements. 58

Figure 13. Principal components analysis plots for loblolly pine, longleaf pine, and shortleaf pine, using individual leaf litter trait data, grouped by burned or unburned status. Different shapes represent different collection locations. Each point represents an individual leaf. 59

Figure 14. Principal components analysis plots for black oak, post oak, scarlet oak, southern red oak, and white oak, using individual leaf litter trait data, grouped by burned or unburned

status. Different shapes represent different collection locations. Each point represents an individual leaf. 60

Figure 15. Principal components analysis plots for mockernut hickory, red maple, sweetgum, and tulip poplar, using individual leaf litter trait data, grouped by burned or unburned status.

Different shapes represent different collection locations. Each point represents an individual leaf. 61

List of Abbreviations

IPCC	Intergovernmental Panel on Climate Change
VWC	Volumetric water content
USDA	U.S. Department of Agriculture
NOAA	National Oceanic and Atmospheric Administration
NCEI	National Centers for Environmental Information
C.I.	Confidence Interval
C.L.	Confidence Limit
SLA	Specific leaf area
SA:V	Surface area to volume
LDI	Leaf dissection index
PCA	Principal components analysis

Chapter 1. How do climate and life-history impact regeneration success of southern pine species?

Abstract

Successful natural regeneration of southern pine species – longleaf pine (*Pinus palustris* Mill.), shortleaf pine (*P. echinata* Mill.), loblolly pine (*P. taeda* L.), and slash pine (*P. elliottii* Engelm.) – is dependent on many interacting factors, including species life-history traits, prevailing climate conditions, competition, and predation. Yet, parts of the regeneration process for these species, specifically surrounding the time between seedfall and germination, are not well understood. To address this, we conducted a germination trial using southern pine species with increasing soil temperature and decreasing soil moisture, collected longleaf pine seeds from trees with known cone counts, and conducted a germination trial using the collected seeds. We found species differences in germination rates, but no significant effect of soil temperature and soil moisture on germination. We observed quadratic relationships between cone production and seed production in longleaf pine, but we did not observe trends between cone production and seed size or germination rate. We did observe a logistic relationship between germination and seed size. Understanding the impacts of soil temperature and moisture on germination rate is important given the context of climate change and common management activities, such as prescribed fire and thinning. Identifying how cone production influences seed production, seed size, and germination rate will provide managers and landowners with a better method of predicting the success of natural regeneration efforts in their longleaf pine stands.

Introduction

Natural regeneration is a complex process that is influenced by many interacting factors, including the life-history of the regenerating species and a range of external factors. Each species

has what is known as a regeneration niche, which is the process of regeneration based on the life-history of that species, including seed production, dispersal, germination, seedling establishment, and recruitment into the overstory (Grubb 1977; Oliver et al. 1996; Clark et al. 1999; Dey et al. 2019). This regeneration niche exists between two planes of regeneration potential, which is determined by the sources of regeneration in the stand for the regenerating species and its competitor species (Johnson et al. 2019), and regeneration success, which depends on external factors such as prevailing environmental conditions (e.g., temperature, light, water), forest structure, regeneration space, and silvicultural practices (Dey et al. 2019). Any of these external factors can limit regeneration success by causing a part of the regeneration niche to fail (e.g., water availability limiting seed production or a late frost killing young seedlings). Understanding the impacts of these factors on the regeneration niche and regeneration success is imperative for improving the natural regeneration of a species or forest.

Climate is an important factor that can impact the regeneration niche and success, specifically germination and early seedling establishment. Suitable temperature and sufficient moisture are crucial to every part of the regeneration process, from seed production through overstory recruitment (Walck et al. 2011; Dey et al. 2019). In a study where longleaf pine (*Pinus palustris* Mill.) seedlings germinated from different seed sources were planted in locations with varied climates across the southeastern U.S., researchers found that growth rate of seedlings originating from seeds collected in south Alabama withstood temperatures up to 1.7 °C higher than their average annual temperature of origin, but did not tolerate temperatures 2.8 °C higher (Wells and Wakeley 1970), which may prove problematic in the face of a warming climate. Furthermore, longleaf pine seedlings in north Florida were found to be negatively impacted by experimental warming in the understory of a longleaf pine forest (Aspinwall et al. 2022).

However, in dry forests, findings suggest that rainfall and therefore soil moisture will likely be more limiting than soil temperature to seed germination in the face of climate change (Dantas et al. 2020). Changes in soil moisture, resulting from predicted changes in temperature and precipitation, are likely to influence other aspects of seed germination as well, such as seed longevity, dormancy release, and presence of soil pathogens (Walck et al. 2011). The interaction of temperature and water availability may also be important, with high temperatures and low moisture potentially having vastly different effects than high temperatures and high moisture. While climate plays a large role in the regeneration process, other external factors are also important.

Competition, seed predation, and prescribed fire may impact natural regeneration as well. Longleaf pine in particular often lacks successful regeneration when faced with extensive competing vegetation (Barnett and Pesacreta 1993), and slash pine (*P. elliottii* Engelm.) and loblolly pine (*P. taeda* L.) experience negative effects on germination and seedling establishment when exposed to allelopathic understory competition (Hollis et al. 1982). Accumulation of leaf litter may limit germination in intervals between fires (Rodríguez-Calcerrada et al. 2011), as exposed mineral soil can improve germination of longleaf pine (Willis et al. 2019, 2021). However, seeds on exposed mineral soil are more vulnerable to seed predators (Willis et al. 2019, 2021). In many areas, managers are returning fire to the landscape, which could improve seedbed conditions for longleaf pine seed germination by removing litter accumulation and competing vegetation. These burns can also lead to increases in soil temperature and decreases in soil moisture (Iverson and Hutchinson 2002), which may limit germination success. A deeper understanding of the external factors influencing seed germination will be important for advancing natural regeneration efforts.

The major southern pine species – longleaf pine, shortleaf pine (*P. echinata* Mill.), loblolly pine, and slash pine – have differing seed traits, growth strategies, and environmental tolerances that may yield different responses to changing climate conditions. Longleaf pine has the largest seeds of the southern pines (Dorman 1976), and the seeds can germinate in less than one week in suitable conditions (Cahalan 1985). Shortleaf pine has the smallest seeds of the southern pines and may be more susceptible to adverse seedbed conditions. Loblolly pine has highly dormant seeds that are greatly affected by weather conditions and soil moisture (Dorman 1976; Cahalan 1985). Slash pine seeds may germinate rapidly with adequate soil moisture conditions (Cahalan 1985). In a study testing germination of the major southern pine species under different temperature and stratification treatments, longleaf pine had better germination in lower temperatures than higher temperatures, and unstratified slash pine was less affected by temperature extremes than shortleaf pine and loblolly pine (Barnett 1979). These temperature differences were air temperatures rather than soil temperatures, which could be warmer than air temperatures under open high light conditions (Loudermilk et al. 2016; Dantas et al. 2020). Longleaf pine and slash pine germination are both affected by moisture stress in lab-based osmotic stress testing (Barnett 1969), but we do not yet know if this holds true with the combination of moisture and temperature stress. Due to the varying life-history and seed traits of the major southern pine species, it is unknown how their regeneration will respond to changing climate conditions.

Cone production, a commonly studied process, plays a large role in the complex process of natural regeneration in pines, but we do not yet understand the relationships between cone production and seed production, seed size, and germination rate. In longleaf pine, cone production is a three-year process, with cone development and ripening occurring in the third

year and seed dispersal occurring in October and November of that year (Boyer 1963a; Pederson et al. 1999; USDA 2008). With cyclical cone production, good mast years with high cone production tend to occur every four to seven years, but there can be wide geographic variation in a single year (Wahlenberg 1946; Boyer 1990; Chen and Willis 2023). Precipitation and average monthly temperatures just prior to key periods of cone development heavily influence cone crop variation, and these climatic factors are hypothesized to determine whether the tree continues to allocate resources for investing in cone and seed production in a given year (Pederson et al. 1999; Chen et al. 2016). These conditions are important factors in the growing season a tree experiences and the climatic stress imposed upon the tree, which may negatively affect bud and cone development. Studies on red pine (*Pinus resinosa* Ait.) show that the species' seed production capacity depends largely on cone size and volume, position in the crown, and number of structurally complete ovules at the time of pollination (Lyons 1956; Dickmann and Kozlowski 1971), but overall cone production and viability of seeds were not recorded. There is a lack of studies in the literature quantifying the relationship between cone production and seed production, size, and germination rate for longleaf pine or other conifers.

Seed size and seed quality are important factors in the germination portion of the natural regeneration process. Seed size is often used as an easily measured indicator of seed quality for many species, with larger seeds having more seed-stored reserves for better seedling establishment in the face of competition (Gross and Werner 1982; Gross 1984; McConnaughay and Bazzaz 1987; Reader 1993; Leishman 2001) or adverse environmental conditions (Leishman and Westoby 1994b; Leishman 2001). Lower quality seeds will be less likely to germinate than higher quality seeds. However, even higher quality seeds may experience other obstacles to natural regeneration, such as unsuitable conditions, seed predation, or competition with other

seedlings and vegetation. We know that longleaf seeds are the least dormant of the southern pine species, able to germinate in around one week under favorable conditions without stratification (Barnett and Pesacreta 1993). However, it is unclear whether seed size and germination are related in longleaf pine.

A major knowledge gap surrounds several crucial aspects of the natural regeneration process of southern pine species, including the influence of soil temperature and soil moisture on seed germination and the relationships between cone production and seed production, seed size, and seed germination. The primary goals of this study were to 1) evaluate the impacts of shifting environmental conditions on southern pine seed germination and 2) explore the relationship between cone production and the quantity and quality of seeds produced in longleaf pine. The more specific objectives of this study were as follows: 1) determine the impacts of increased temperature and reduced soil moisture on seed germination rates of the major southern pine species, 2) determine the relationship between cone production and seed production across individual mature longleaf trees, 3) determine whether cone production and seed size characteristics are related across mature longleaf trees, and 4) determine whether seed germination rates vary as a function of cone production across mature longleaf trees.

Our hypotheses were as follows, relating to the previous objectives. Germination rates would generally decrease with increasing soil temperature and decrease with decreasing soil moisture, and longleaf pine seeds would have the highest germination rates under higher soil temperature and lower soil moisture conditions due to larger seed size compared to the other southern pine species, which would increase seed-stored reserves. Trees with higher cone counts would produce more total seeds and a higher proportion of developed seeds than trees with lower cone counts. Seed size metrics – including seed length, width, and mass – would decrease with

increasing cone counts due to the seed size/number tradeoff that is generally found across many species and growth forms (Leishman 2001); furthermore, we propose that seeds with higher masses would have higher germination rates than seeds with lower masses because of increased seed-stored carbohydrates allowing for better seedling establishment (Leishman and Westoby 1994b; Leishman 2001). Germination rates of collected seeds would increase with increasing cone production up to a point (one standard deviation above mean cone production across all sites for all years) and then level off or decline above that level of cone production. Gaining a better understanding of the influences of temperature and moisture on germination will provide more insight into the intermediate stages of the regeneration process across multiple species, while our study investigating the relationships between cone production and seed production, size, and germination will tell us more about the early stages of the regeneration process.

Methods

Soil Temperature and Soil Moisture Experiment

We purchased longleaf pine (sourced from Florida, U.S.A.), shortleaf pine (*P. echinata* Mill., sourced from Arkansas, U.S.A.), loblolly pine (*P. taeda* L., sourced from Arkansas, U.S.A.), and slash pine (*P. elliotii* Engelm., sourced from Georgia, U.S.A.) seeds from Sheffield's Seed Company (Locke, New York, U.S.A.). On 31 March 2022, we planted these seeds in trays in a greenhouse in Auburn, Alabama, U.S.A., and exposed them to increased temperature and decreased soil moisture conditions for 60 days. The greenhouse temperature was set to 20 °C, but ambient temperature and light varied with diurnal cycles. The substrate was a peat-based planting medium (PRO-MIX Premium Moisture Potting Mix, Premier Horticulture

Inc., Quakertown, Pennsylvania, U.S.A.). An additional “no seed” treatment was included to allow empty cells for monitoring soil temperature and moisture.

There were two temperature conditions: one that used the ambient temperature and one with an increased soil temperature of approximately 3 °C compared to the ambient greenhouse air temperature. We chose 3 °C based on the Intergovernmental Panel on Climate Change’s (IPCC) predicted increases in mean global air temperature by the end of the 21st century (Meehl et al. 2007). The increased soil temperature treatment was implemented using heating mats (VIVOSUN 48” x 20.75” Seedling Heat Mat and Digital Thermostat, VIVOSUN, Ontario, California, U.S.A.) placed under the trays and set to the appropriate temperature. Heating mats are commonly used in horticultural experiments (Campbell et al. 2020) and have been used in seedling experiments for thornscrub forest species (Luera et al. 2021). Initially, the heating mats were set to 23 °C because that was 3 °C higher than the temperature setting for the greenhouse, which was 20 °C. On 4 April 2022, it became clear that 23 °C was not high enough to create a 3 °C soil temperature difference between the increased and ambient temperature treatments, so we increased the heating mats to 29 °C to create a soil temperature increase of 3 °C from the average soil temperature of the ambient temperature treatment trays. There were two soil moisture conditions: a high soil moisture treatment that was watered three times per week and a low soil moisture treatment that was watered once per week. Both soil moisture treatments began with saturated soil at the time of planting, and we watered the trays by hand using a sprayer head and approximately equal pressure and time on each tray according to the treatment watering schedule.

Forty-two seeds of each species were used in each of the following treatments: 1) ambient soil temperature and high soil moisture, 2) ambient soil temperature and low soil

moisture, 3) increased soil temperature and high soil moisture, and 4) increased soil temperature and low soil moisture. The layout of the experiment in the greenhouse is shown in Figure 1, with the experiment occurring all on one greenhouse bench. Rows of four trays were randomly assigned to a soil temperature treatment. Each soil temperature treatment row was divided in half and the halves randomly assigned to a moisture treatment. Within each tray, the five species treatments were randomly assigned to a row of cells.

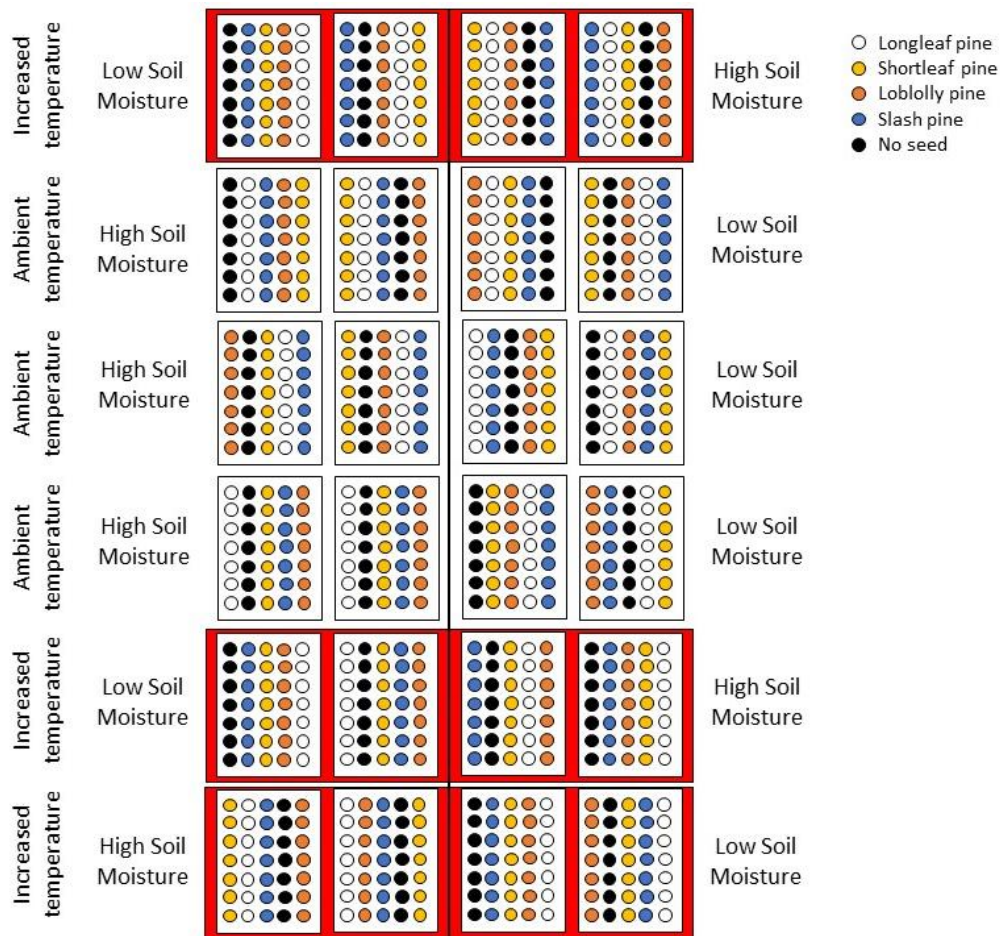


Figure 1. Experimental design for greenhouse germination trial. Heating mats for increased temperature treatments are represented by red rectangles. Trays in each moisture treatment are divided by the black line down the center of the diagram. The species treatments, shown by colors in each cell, are as follows: longleaf pine (*Pinus palustris* Mill.), shortleaf pine (*P. echinate* Mill.), loblolly pine (*P. taeda* L.), slash pine (*P. elliottii* Engelm.), and no seed (for temperature and moisture monitoring). The experiment occurred on one bench in the greenhouse, with the top of the figure representing the end of the bench closest to the window and the bottom of the figure representing the end of the bench closest to the center of the greenhouse.

On 31 March 2022, we planted the seeds at a depth of approximately one seed length with the end of the seed that would have been attached to the wing oriented upwards. We monitored and recorded soil temperature, soil moisture, and germination three times per week over the course of 60 days, with the germination trial ending on 30 May 2022. Soil temperature (°C) and moisture (% volumetric water content, VWC) were recorded prior to any watering activities, with a soil probe (3-in-1 Digital Soil EC Moisture Temperature Meter, YY-1000, Yieryi, Shenzhen, Guangdong Province, China). These values were used to calculate treatment average soil temperature and treatment average soil moisture. Germination was considered complete when the radicle, hypocotyl, and cotyledons emerged (Barnett et al. 1999).

Study Sites for Longleaf Pine Seed Collection

Study sites (Figure 2) for longleaf pine seed collection included the Jones Center at Ichauway (Newton, Georgia, U.S.A.), Blackwater River State Forest (Milton, Florida, U.S.A.), and Eglin Air Force Base (Fort Walton Beach, Florida, U.S.A.). All soil information and climate information were sourced from the U.S. Department of Agriculture (USDA) Web Soil Survey and the National Oceanic and Atmospheric Administration (NOAA) National Centers for Environmental Information (NCEI) U.S. Climate Normals data. The Jones Center at Ichauway (Jones Center) site was a 100-year-old, open longleaf pine stand with wiregrass (*Aristida stricta* Michx.) understory. The primary soil type at the Jones Center was Suffolk loamy fine sand on zero to two percent slopes. The average annual temperature at the Jones Center is 19.2 °C, with a minimum temperature of 3.8 °C and a maximum temperature of 32.7 °C. Average annual precipitation at the Jones Center is 125.7 cm. The Blackwater River State Forest (Blackwater) site was a 72-year-old, open longleaf pine stand with an understory consisting primarily of

southern red oak (*Quercus falcata* Michx.) and longleaf pine tree seedlings; blackberries (*Rubus* spp. L.), winged sumac (*Rhus copallinum* L.), and gallberry (*Ilex glabra* A. Gray) shrubs; and wiregrass and broomsedge bluestem (*Andropogon virginicus* L.) the most common grasses (Brockway and Outcalt 2017). The primary soil type at the Blackwater site was Troup loamy sand on zero to five percent slopes. The average annual temperature at Blackwater is 19.2 °C, with a minimum temperature of 3.8 °C and a maximum temperature of 32.8 °C. Average annual precipitation at Blackwater is 161.0 cm. At Eglin Air Force Base (Eglin), there was a 70-year-old longleaf pine stand with grassy and shrubby understory dominated by gallberry, dwarf palmetto (*Sabal minor* Jacq.), and yaupon (*Ilex vomitoria* Aiton). The primary soil type at the Eglin site was Chipley and Hurricane soils on zero to five percent slopes. The average annual temperature at Eglin is 19.4 °C, with a minimum temperature of 3.6 °C and a maximum temperature of 33.5 °C. Average annual precipitation at Eglin is 161.5 cm.

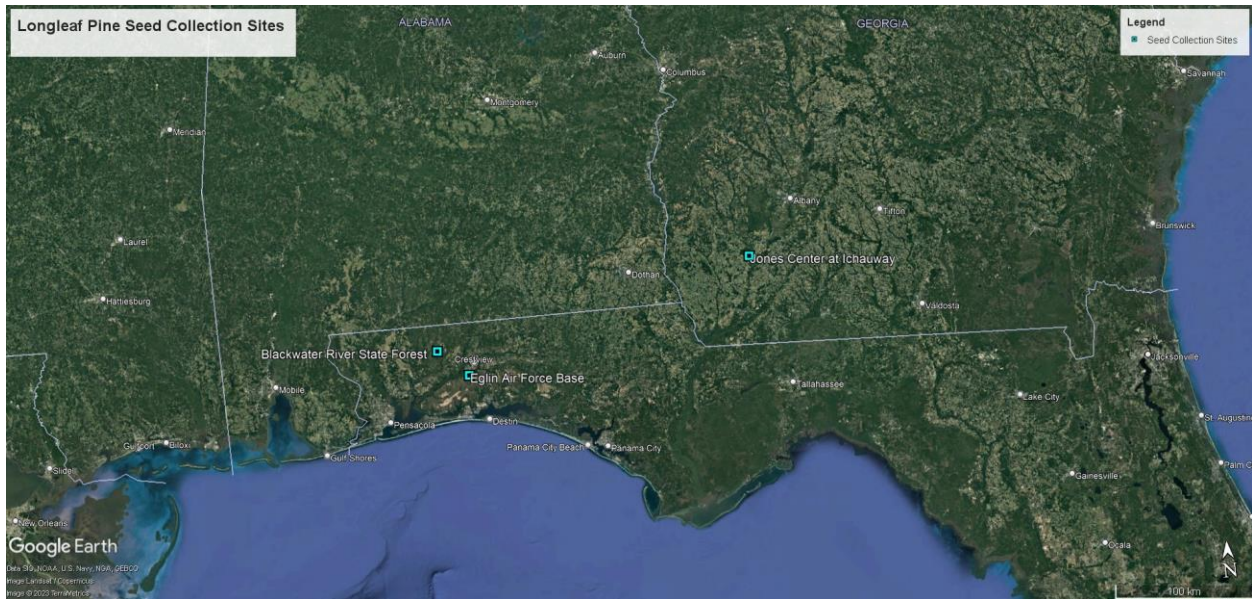


Figure 2. Longleaf pine seed collection sites, including Blackwater River State Forest (Milton, Florida, U.S.A.), Eglin Air Force Base (Fort Walton Beach, Florida, U.S.A.), and the Jones Center at Ichauway (Newton, Georgia, U.S.A.).

Longleaf Pine Seed Collection

We conducted two rounds of longleaf pine seed collection, one during fall 2021 and one during fall 2022. During fall 2021, we conducted an initial round of seed collection under 10 longleaf pine trees with known cone counts indicative of a mast year (based on data from Willis and Brockway (2021)). Seed was collected at only one site (Jones Center) using a seed trap design similar to Scholtens (1979). Our seed traps were 0.5 m x 0.5 m (0.25-m²) and mounted on wooden legs. They consisted of a bottom frame with legs and a top frame secured to the bottom frame using zip ties. In fall 2022, we expanded our collection efforts to three sites using a reconfigured seed trap design of lighter construction that expanded the collection area by 2.75 m² per trap and simplified trap transport. Our new trap design was a single 1 m x 3 m PVC pipe frame with four 0.5-m legs. A basket made of crinoline mesh fabric was attached to the frame using zip ties, and 2.54 cm mesh deer fencing was zip tied across the top of the frame to act as a lid. The legs of the trap allowed the trap to be anchored in the ground and prevented the trap from being moved by high winds. The two trap designs are shown in Figure 3. We standardized the number of seeds collected each year on a trap area basis.



Figure 3. Examples of 0.25-m² seed traps from the 2021 longleaf pine seed collection and 3-m² seed traps from the 2022 longleaf pine seed collection.

For the initial seed collection at the Jones Center, we placed 10 seed traps under each tree, evenly spaced at approximately the midpoint between the bole and the dripline. We installed the traps on 15–16 October 2021 and emptied the traps approximately every two weeks through 4 December 2021, at which point we collected the seed traps from the field. This date range was chosen based on the timing of longleaf pine seedfall found in (Boyer 1963a), where seedfall began in October, peaked in November, and dwindled in December. Collected seeds were held in cold storage (0 to 4 °C) until later experimental use.

The second round of seed collection was conducted at the Jones Center at Ichauway, Blackwater River State Forest, and Eglin Air Force Base. The same 10 trees from the fall 2021 collection were used at the Jones Center for the fall 2022 collection. At Blackwater River State Forest, 10 trees were used for seed collection, and 10 trees were used at Eglin Air Force Base. On 17–18 October 2022, we installed three seed traps per tree, evenly spaced at approximately

the midpoint between the bole and the dripline, at Eglin Air Force Base and the Jones Center at Ichauway. The seed traps at Blackwater River State Forest were installed on 22 October 2022, using the same methods. We collected seeds approximately every two weeks until 11–12 December 2022, at which point we removed the traps and stored them for future use. Collected seeds were held in cold storage (0 to 4 °C) until later experimental use.

After each round of seed collection, we sorted the collected seeds into categories based on visual inspection and counted the number of seeds in each category. The categories included 1) developed seeds, which were seeds that had fully developed and were fit for use in a germination trial; 2) undeveloped seeds, which were wings that did not show signs of developing into a viable seed; and 3) damaged seeds, which were developed seeds that were damaged from attempted predation or other means. Examples of seeds in each category are shown in Figure 4.



Figure 4. Examples of seeds from the different categories of collected seeds. Developed seeds are fully developed and fit for use in a germination trial. Undeveloped seeds are wings that did not show signs of developing into a viable seed. Damaged seeds are developed seeds that were damaged through attempted predation or other means.

Longleaf Pine Seed Size

We measured length (mm), width (mm), and mass (g) of collected seeds to determine if cone production influences seed size and if seed size influences germination rate. We also

measured total length (including the wing), wing width, and total mass (including the wing). Measurements were taken on all developed seeds for the 2021 collection and only on the seeds to be used in the germination trial (see below) for the 2022 collection. Length and width were measured to the nearest 0.01 cm using calipers, and mass was measured in grams to the nearest 0.0001 g.

Collected Longleaf Pine Seeds Germination Trial

We planted collected seeds in propagation trays and placed them in a growth chamber (Percival Model E41L1, Percival Scientific, Inc., Perry, Iowa, U.S.A.; 20 °C; 16 hr light; 8 hr dark) and tracked their germination for 42 days (AOSA 1993). The first germination trial began on 2 February 2022 and ended on 16 March 2022. We randomly selected six developed seeds from each tree for the trial, proportionally distributed across the collection dates from available seeds. Six seeds were used for each tree because the lowest number of developed seeds collected from a tree was six. Because one of the trees did not have any cones on it, we did not use any seeds collected from traps under it, as they could not have been produced by that tree. The second germination trial began on 11 February 2023 and ended on 25 March 2023. We used 30 developed seeds from each tree and selected the seeds for the trial randomly, proportionally distributed across the collection dates from available developed seeds. For both germination trials, seeds were checked at least every three days for signs of germination (i.e., emergence of the radicle, hypocotyl, and cotyledons (Barnett et al. 1999)).

Data Analysis

Germination results from the greenhouse germination trial were analyzed using a split-split plot design to account for the soil temperature, soil moisture, and species treatment factors and their potential interactions. The first split was the soil temperature treatment, and the second split was the soil moisture treatment, with 14 seeds of each species experiencing the same soil temperature and soil moisture treatment combination. We included fixed effects for soil temperature treatment, soil moisture treatment, and species in our model as well as their interactions. The position of the trays in the greenhouse led to the seeds being exposed to differing light levels ($144.6 \mu\text{mol}/\text{m}^2\text{s}$ in center of greenhouse – $824.0 \mu\text{mol}/\text{m}^2\text{s}$ by the windows), which influenced soil temperature and soil moisture fluctuations, so we also included a random effect in the model to account for the location of each experimental unit. We also accounted for differences in the germination cuts of the species by inflating the percent germination values by a value proportional to the deficit of the germination cut. For example, the germination cut for loblolly pine was 98% (Sheffield's Seed Company, Locke, New York, U.S.A.), so we inflated percent germination by 2% of the current percent germination at each measurement point to account for seeds that would not have germinated due to initial non-viability.

For the longleaf pine study, we conducted several analyses. The relationships between cone production and total seeds collected/ m^2 trap area, total developed seeds collected/ m^2 trap area, and total undeveloped seeds collected/ m^2 trap area were analyzed at the tree level using both linear and quadratic regression models to evaluate the relationship between number of cones produced and number of seeds produced. To identify trends among cone production level and seed size, we analyzed the relationship between cone production and seed size at the tree level using a linear regression model. The relationship between seed size and germination was

analyzed at the individual seed level using a binomial logistic generalized linear model. The relationship between cone production and germination rate was analyzed at the tree level using a linear regression model. For the germination versus cone production analysis, we used a complete randomized block design to account for differences in light availability (16.5 $\mu\text{mol}/\text{m}^2\text{s}$ on the bottom shelf – 730.6 $\mu\text{mol}/\text{m}^2\text{s}$ on the top shelf) and moisture retention among the three shelves, ensuring that 10 seeds from each tree were on each shelf, distributing the differences in light and moisture across all the trees evenly. We also only watered the trays as needed, or when the surface of the soil felt dry to the touch, to avoid oversaturating the lower trays. We did not observe significant differences in germination across the trays due to these conditions. We removed one tree at Blackwater from all analyses as an outlier because of an increased number of undeveloped seeds collected due to cones falling into the trap.

Results

Soil Temperature and Soil Moisture Experiment

In the greenhouse germination trial, we did not observe significant differences in final percent germination (Figure 5) between soil temperature and soil moisture treatments (all p -values > 0.05), but we did observe significant differences in final percent germination between species ($p < 0.0001$). On the final day of the germination trial, longleaf pine had 17.07% ($\pm 5.47\%$; $\pm 95\%$ C.I.) higher final percent germination than loblolly pine ($p < 0.0001$). Shortleaf pine had 25.10% ($\pm 5.47\%$; $\pm 95\%$ C.I.) higher final percent germination than loblolly pine ($p < 0.0001$). Slash pine had 43.17% ($\pm 5.47\%$; $\pm 95\%$ C.I.) higher final percent germination than loblolly pine ($p < 0.0001$). Shortleaf pine had 8.04% ($\pm 5.47\%$; $\pm 95\%$ C.I.) higher final percent germination than longleaf pine ($p = 0.0009$). Slash pine had 26.10% ($\pm 5.47\%$; $\pm 95\%$ C.I.)

higher final percent germination than longleaf pine ($p < 0.0001$). Slash pine had 18.06% ($\pm 5.47\%$; $\pm 95\%$ C.I.) higher final percent germination than shortleaf pine ($p < 0.0001$). Percent germination over time by species is shown in Figure 6.

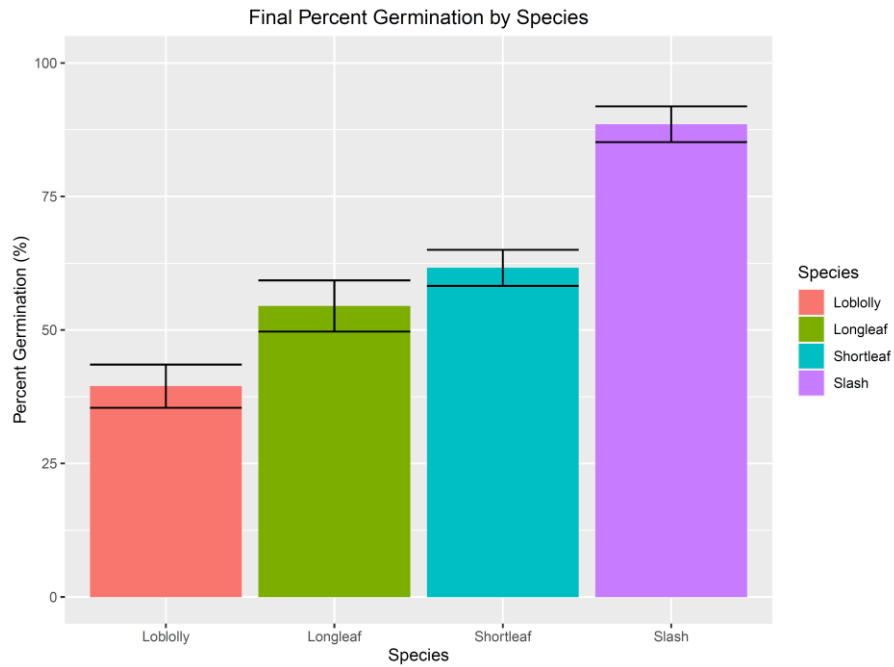


Figure 5. Percent germination (\pm SE) for loblolly, longleaf, shortleaf, and slash pine seeds on the final day of the greenhouse germination trial.

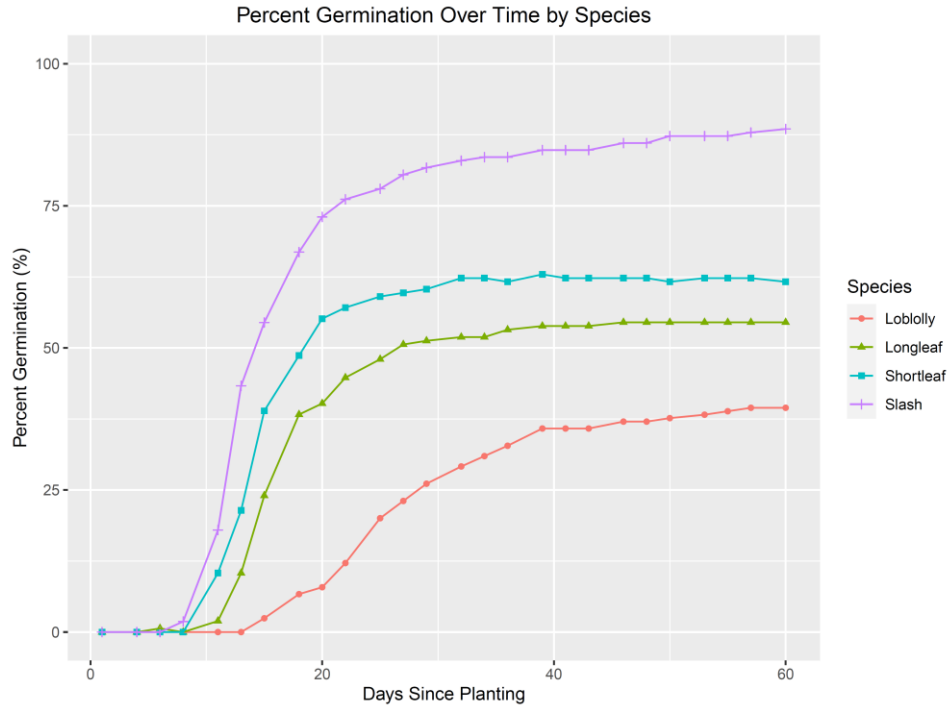


Figure 6. Percent germination over time of loblolly, longleaf, shortleaf, and slash pine seeds throughout the 60-day greenhouse germination trial.

Longleaf Pine Seed Collection Results

Total seeds, developed seeds, and undeveloped seeds collected/m² trap area all increased with increasing cone production before declining (Figure 7). These relationships were better represented by quadratic models than by linear models (Table 1). Total number of seeds collected/m² trap area from each tree increased with cone production up to approximately 123 total seeds collected/m² trap area at 107 cones per tree before declining (Figure 7A). Total number of collected seeds includes both developed and undeveloped seeds. The total number of developed seeds collected also increased with cone production up to approximately 75 developed seeds collected/m² trap area at 109 cones per tree and then began to decline at the higher cone counts (Figure 7B). Total number of undeveloped seeds collected increased with cone production up to approximately 42 undeveloped seeds collected/m² trap area at 104 cones per tree and then

began to decline (Figure 7C). Interestingly, the proportion of developed seeds/m² trap area to total seeds collected/m² trap area and the proportion of undeveloped seeds/m² trap area to total seeds collected/m² trap area remained relatively constant across the range of cone production. The 2021 collection occurred during a poor cone year, but we included the data here to provide more data for the analysis.

Table 1. Comparison of linear model and quadratic model for total seeds, developed seeds, and undeveloped seeds collected/m² versus cone count (x).

Response Variable	Model	Equation	R²	P-value	AIC
Total Seeds Collected/m²	Linear	0.3581x + 63.7861	0.1638	0.0117	388.74
	Quadratic	-0.007094x ² + 1.523476x + 41.518374	0.3668	0.0003365	380.68
Developed Seeds Collected/m²	Linear	0.22533x + 38.42983	0.1513	0.0158	357.12
	Quadratic	-0.004139x ² + 0.905299x + 25.437299	0.3125	0.00142	351.62
Undeveloped Seeds Collected/m²	Linear	0.11445x + 21.58502	0.1357	0.0229	388.74
	Quadratic	-0.0025992x ² + 0.5414209x + 13.4265966	0.3567	0.0004437	380.68

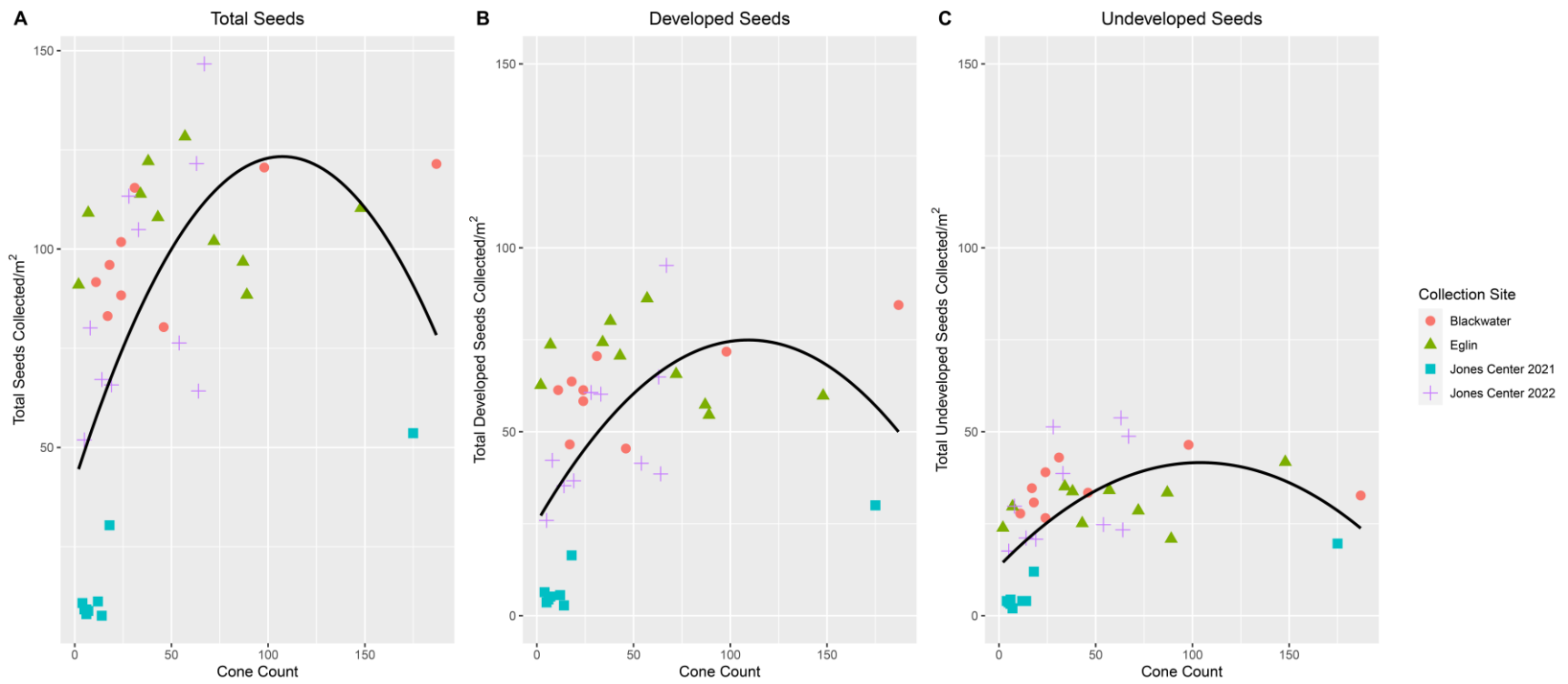


Figure 7. Total number of seeds collected/m², developed seeds collected/m², and undeveloped seeds collected/m² versus number of cones produced on each tree at Blackwater River State Forest (2022), Eglin Air Force Base (2022), and the Jones Center at Ichauway (2021 and 2022).

Longleaf Pine Seed Size Results

There were no significant relationships between any of the seed size metrics and cone production, so we have reported the overall mean and standard error for seed length, seed width, total length, wing width, total mass, and seed mass in Table 2. While cone production did not impact seed size, we did find that probability of germination improved with increasing seed size (Figure 8). For every 0.01 g increase in seed mass, we observed that a longleaf pine seed is 2.11 (1.94 – 2.32; 95% C.L.) times more likely to germinate ($p < 0.0001$).

Table 2. Mean (SE) seed size measurements for longleaf pine seeds collected from Blackwater River State Forest (2022), Eglin Air Force Base (2022), and the Jones Center at Ichauway (2021 and 2022).

Seed Size Measurement	Mean (SE)
Seed Length (cm)	1.03 (0.00)
Seed Width (cm)	0.68 (0.00)
Total Length (cm)	4.19 (0.02)
Wing Width (cm)	0.91 (0.00)
Total Mass (OD g)	0.0725 (0.0010)
Seed Mass (OD g)	0.0629 (0.0009)

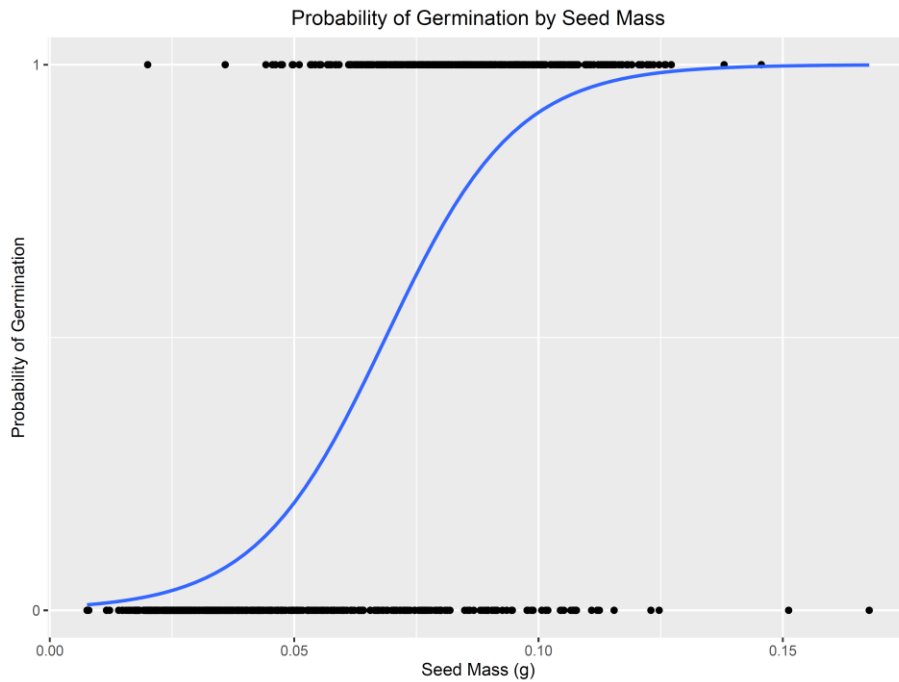


Figure 8. Germination by seed mass (g) of longleaf pine seeds (black points) and predicted germination (blue line) for a given seed mass based on a logistic model of the germination trial results.

Collected Longleaf Pine Seeds Germination Trial Results

We observed no definite relationship between cone production and seed germination in longleaf pine seeds (Figure 9). Cone count does not appear to affect seed germination. However, seed size, as previously mentioned, may impact seed germination.

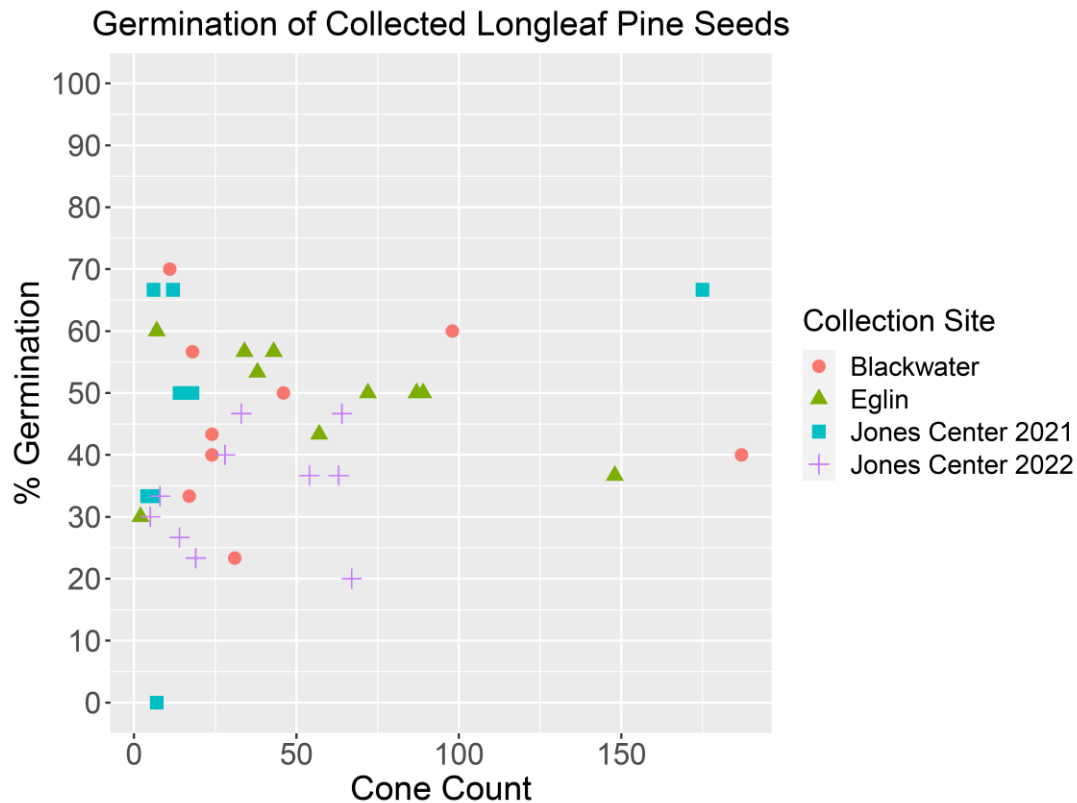


Figure 9. Percent germination of longleaf pine seeds collected from Blackwater River State Forest (2022), Eglin Air Force Base (2022), and the Jones Center at Ichauway (2021 and 2022) versus number of cones produced per tree.

Discussion

Regeneration of southern pine species is a complex process in which success depends on many interacting factors from species life-history traits (Dey et al. 2019) to external conditions such as climate (Walck et al. 2011; Dey et al. 2019), predation (Willis et al. 2019, 2021), and competition (Hollis et al. 1982; Barnett and Pesacreta 1993). We found that soil temperature and

soil moisture conditions did not influence germination of southern pine seeds as we expected, but species differences in germination did occur. In longleaf pine, we found that seed production was quadratically related to cone production rather than the linear relationship we expected, but cone production had no significant impact on seed size or germination, contrary to our hypotheses. However, seed size exhibited a logistic relationship with probability of germination, which was expected. Overall, we found that species and seed size had the largest impact on germination, and that seed production is correlated with cone production.

Soil Temperature and Soil Moisture Experiment

In the greenhouse germination experiment, we found that our created soil temperature and soil moisture treatments did not have a significant impact on the germination, contrary to our hypothesis; however, we observed significant differences in germination between species. These species differences may be due to differences in temperature and moisture tolerances of the species (Barnett 1969, 1979; Dorman 1976; Cahalan 1985), or they may stem from other factors, such as seed quality (Leishman and Westoby 1994b) or seed source climate (Wells and Wakeley 1970). For example, the seed sources may have impacted the seeds' ability to tolerate differing temperature and moisture conditions (Wells and Wakeley 1970). With loblolly pine and shortleaf pine seeds from Arkansas, longleaf pine seeds from Florida, and slash pine seeds from Georgia, seeds from warmer climates may have been better able to tolerate increased soil temperatures than seeds from cooler climates. Wells and Wakeley (1970) found that the growth rate of longleaf pine seedlings originating from seeds collected in south Alabama withstood temperatures up to 1.7 °C higher than their average annual temperature of origin, but did not

tolerate temperatures 2.8 °C higher. This study focused on seedlings rather than germination, but perhaps germination follows a similar pattern with seeds from sources with varying climates.

Seed dormancy and potential differences in germination cues of the species may have influenced the species differences we observed. For example, longleaf pine is the least dormant of the southern pines and has a thinner seed coat that allows faster imbibition of moisture, whereas loblolly pine is the most dormant of the southern pines and has a thicker seed coat that slows down imbibition of moisture (Barnett 1991). Loblolly pine's lower germination may be due to its higher dormancy and slower moisture absorption for germination. Slash pine has a seed coat that is intermediate to longleaf and loblolly pine seeds (Barnett and Varela 2003), and slash and shortleaf pine are both commonly exposed to cold stratification practices, or allowing seeds to imbibe moisture and then chilling them at 1 – 4 °C for a period of time, to increase germination and break dormancy (Barnett 1993). Slash pine may have had the ideal seed coat and dormancy characteristics to germinate more successfully under the conditions of our treatments compared to the other species.

A potential reason for the lack of observed effects of soil temperature on germination may have been that the conditions we created with our treatments were not outside of the normal range of field germination conditions for the species. For our soil temperature treatments, we set the greenhouse temperature to 20 °C, which resulted in a mean soil temperature of 25.7 °C (± 0.3 °C) for the ambient soil temperature treatment. We set the heating mats to 29 °C, which was 3 °C higher than the mean temperature from the ambient soil temperature treatment on the first day and resulted in a mean soil temperature of 27.7 °C (± 0.4 °C) for the increased soil temperature treatment. IPCC future climate predictions show a predicted 3 °C increase in the mean global average air temperature by the end of the 21st century if atmospheric CO₂ doubles, compared to

1980 – 1999 temperatures (Meehl et al. 2007), which is why we chose 3 °C as our intended temperature increase. In May, soil surface temperature in an open longleaf sandhill ecosystem can range from 35.8 °C – 55.3 °C at midday under varying light conditions and canopy openness (Loudermilk et al. 2016). This is a different time of year than when we might expect southern pine seeds to germinate in the field, but perhaps our soil temperature treatments did not reach temperatures that would have truly stressed the seeds during the times they would typically germinate.

We did not observe a significant effect of soil moisture on germination, and it may be due to the levels of soil moisture in our treatments. While our soil moisture treatments were significantly different from each other, both had rather low mean soil moisture values (High soil moisture = 23.4% VWC \pm 0.7% VWC, Low soil moisture = 14.0% VWC \pm 0.7% VWC). Some studies have shown that rainfall (and thus soil moisture) may be more limiting to germination than soil temperature in dry forests (Dantas et al. 2020), but we may not be seeing that here due to the low soil moisture values of both treatments. In the field, differences in soil moisture have been observed among uncut pine savanna and gaps of varying sizes, but no significant patterns in timing of differences or consistent correlation between soil moisture and gap size were found (McGuire et al. 2001). The soil moistures observed in the McGuire et al. (2001) study were also low (all below 20% gravimetric soil moisture content), meaning that our soil moisture treatments may have reflected similar conditions to the field. Extreme drought conditions would likely be even lower than our low soil moisture treatment values, since 0.06 m³ m⁻³ VWC has been used as a target soil moisture in a previous drought response study (Blackman et al. 2019). Prescribed fire has been found to increase soil temperature and decrease soil moisture (Iverson and Hutchinson 2002), but some have found this effect to be small (Hutchinson et al. 2005), which

may not be substantial enough to limit germination of our studied species. Another reason why our soil moisture treatments may not have impacted germination is because, despite significant differences in average soil moisture between treatments, there was a lack of consistent soil moisture differences throughout the experiment. Initial soil moisture values were similar between the treatments, with soil moisture values diverging more as the experiment progressed, whereas most germination occurred during the first half of the monitoring period. If this experiment were to be repeated, increasing the watering of the high moisture treatment and adding additional soil moisture treatments to form a gradient may be necessary to achieve soil moisture differences that accurately reflect moisture stress levels for these species. Implementing the treatments prior to planting the seeds to provide more consistent treatment differences throughout the monitoring period may also allow better insight into the impacts of soil moisture on germination.

Other factors that may influence germination include light availability and soil type. Light consistently affects germination of dormant seeds (Cohen et al. 2004). Differing light levels, and their impact on soil temperature and moisture, are common in forests with gaps having higher light levels than areas with a closed canopy, and this heterogeneity in light availability and its effects on microclimate conditions can be challenging for seed germination and seedling establishment (Bliss and Smith 1985; Leishman and Westoby 1994a; McGuire et al. 2001). Soil type is another factor that may also impact seed germination and seedling survival in the field that we did not account for in our experiment. Our experiment used a peat and perlite potting mix that is likely better at retaining moisture than the soil that would be found where these species naturally occur. Exposure to a litter layer or bare mineral soil that would be found in their native habitat could yield different results (Ahlgren and Ahlgren 1981). A field experiment attempting to germinate these species in their native soil types may be beneficial to

build on our results. Alternatively, adding a soil type treatment to the greenhouse experiment may be a simpler way of incorporating differences in native soil type in a future experiment.

Longleaf Pine Seed Collection

We observed that total seeds, developed seeds, and undeveloped seeds collected/m² trap area all increased with cone production up to around 100 cones/tree before declining in a quadratic relationship. Our observed results partially supported our hypotheses, in that seed production increased with increasing cone production, but we did not predict the leveling off and decline of seed production. Our hypothesis that the proportion of developed seeds would increase with cone production was not supported, with the proportion of developed seeds to total seeds collected/m² trap area remaining relatively constant across the range of cone counts. This suggests that, while the number of seeds produced may follow a quadratic curve in relation to cone production, longleaf pine trees may remain consistent in the proportion of potentially viable and nonviable seeds produced regardless of cone production.

We collected seeds from trees used for annual longleaf pine cone counts. These trees were considered good seed trees and were in relatively open stands (Willis and Brockway 2021). There were other trees nearby, but the average basal area of surrounding trees that were within 20 m of a collection tree is 0.89 m²/ha (± 0.08 m²/ha), compared to the average basal area of the individual collection trees (0.17 ± 0.04 m²/ha). While we have no way of knowing for sure that the seeds collected are from our collection trees, we accounted for trees that are within a typical dispersal distance of 20 m or less (Grace et al. 2004; Fan et al. 2021). Based on one tree from 2021 where we caught 3 total seeds/m² trap area under a tree with no cones on it and the basal

area of possible interfering trees, we estimated that the average number of seeds caught under a single tree from interfering trees was $16 (\pm 8)$ seeds/m².

Collection is highly influenced by the quality of the cone year and the size of the collection area. Our first year of collection occurred during a poor seed year, and the traps used covered only 2.5 m² per tree. We collected very few seeds in 2021 compared to our second collection year in 2022, when it was a much better cone year and our traps covered 9 m² per tree. Based on extrapolation of our seed collection data, an average tree may produce a total of around 5,039 developed seeds, or 120 seeds per cone if it is an average cone producer. The topic of cone production has been studied extensively (Chen et al. 2016, 2017, 2021; Chen and Willis 2023) and is often used as a substitute for seed production, but there has been a lack of studies on the production of seeds themselves. Further research on this aspect of longleaf pine regeneration could include a wider geographic range of seed collection efforts and cone sampling and dissection to see how many seeds are produced by a single cone.

Longleaf Pine Seed Size

While there were no significant relationships between measured seed size metrics and cone production (contrary to our hypothesis), seed size significantly affected the likelihood of germination, supporting our hypothesis. This suggests that the number of cones produced by a tree does not influence the size of the seeds produced by the tree, which would allow even poor cone producers the potential for successful natural regeneration. The seed-number-seed-size tradeoff suggests that a higher number of seeds produced will result in a smaller seed size (Leishman 2001), but this did not appear to be the case for longleaf pine. The paper refers more to this tradeoff as an interspecific comparison, so it may not be true within a single species. Cone

production and seed production are both reproductive efforts, so there may not be a tradeoff between these two processes. Furthermore, cone production is a three-year process, with the bud year, the flower year, and seed year (Boyer 1990; Pederson et al. 1999). Cones are initially produced during the second (flower) year, but fertilization does not occur for seed production until the third (seed) year (Boyer 1990; Pederson et al. 1999). This suggests that the lack of relationship between seed size and cone production could be due to the phenology of the cone/seed production process. Our findings support previous findings in other species that larger seed size tends to lead to higher germination success and seedling establishment (Gross 1984; Leishman and Westoby 1994a, b; Leishman et al. 2000). The consistency of seed size across cone production levels suggests that longleaf pines may focus more resources on the quality of the seeds in the cones rather than the number of cones produced.

Collected Longleaf Pine Seeds Germination Trial

In the growth chamber germination trial, we did not observe a relationship between cone production and seed germination in longleaf pine. This is likely due to the consistency of seed size across the range of cone production having a stronger impact on seed germination than the number of cones on a tree. Upon extrapolation of our seed collection and seed germination data, an average cone producing tree could potentially regenerate 2.14 seedlings/m². This number is less than the 3.06 seedlings/m² suggested by (Boyer 1963b), but our estimate is limited by our trap placement under the crown of the parent tree that did not account for seeds falling past the dripline of the tree. Longleaf seed dispersal is typically greatest within 18 m of the bole of the tree (Grace et al. 2004; Fan et al. 2021), so our estimate is missing the seeds that fell between the dripline and the 18 m mark.

Conclusions

Soil temperature and soil moisture do not seem to affect germination as much as species differences impact germination. Total number of seeds, developed seeds, and undeveloped seeds exhibit a quadratic relationship with cone production, but the proportion of developed and undeveloped seeds remains relatively constant across the range of cone production. Seed size does not vary across the range of cone production, but seed mass significantly impacts seed germination in longleaf pine. Cone production does not influence seed germination in longleaf pine. These results can help land managers make more informed decisions about species selection when regenerating a stand and timing of management actions around cone production. Further research is needed to continue to fill the knowledge gap surrounding the time between seedfall and seedling establishment in longleaf pine.

Chapter 2. Comparison of leaf litter traits across species, regional/climatic, and burn frequency gradients in the southeastern United States

Abstract

Leaf litter is an important fine fuel source and driver of flammability in forest systems, and leaf litter traits are often measured as a representation of a species' flammability. Although previous research has been conducted to test flammability of individual species and mixed species leaf litter, there is a lack of comprehensive studies that consider the relationship between leaf litter traits, flammability, and environmental conditions across many species. To further our understanding of forest flammability, we conducted a study, based in upland forests of the eastern United States, that explored the relationship among leaf litter traits associated with flammability across a gradient of pyrophytic to mesophytic species sampled across a regional/climatic gradient and in both burned and unburned areas. We collected leaf litter of 12 different species from 12 locations in the eastern United States, with burned and unburned sites at each location, and measured common leaf litter traits (e.g., leaf curl, leaf thickness). Due to the highly correlated nature of the leaf litter traits measured, we analyzed the data using a principal components analysis and found that the two main principal components were significantly affected by species and location, with significant interactions between species and burn status, species and location, burn status and location, and species by burn status by location. We observed diverse intraspecific variation in leaf litter traits by burn status, location, and their interaction. Increased understanding of forest flammability can help land managers effectively return prescribed fire to fire-dependent ecosystems undergoing mesophication as well as better understand mechanisms of flammability that influence wildfire intensity.

Introduction

Leaf litter is an important fuel source for prescribed fire and wildfires in forest systems; thus, understanding leaf litter flammability will ultimately allow us to better predict overall forest flammability and fire behavior. Flammability is defined as the combined ignitibility, sustainability, combustibility, and consumability of a fuel (Anderson 1970; White and Zipperer 2010). Each of these aspects of flammability is associated with common measurements recorded during experimental burns: ignitibility is reflected by time-to-ignition; sustainability is reflected by the fire's rate of spread; combustibility is reflected by fire intensity or rate of burning; and consumability is reflected by the proportion of fuel consumed by the fire (Anderson 1970). Numerous studies have been conducted on how tree leaf litter traits relate to flammability (Engber and Varner 2012; Babl et al. 2020; McDaniel et al. 2021; Varner et al. 2021; Kane et al. 2022), what aspects of flammability are influenced by each trait (Engber and Varner 2012; Varner et al. 2015; Grootemaat et al. 2017), and the relative importance of traits to understanding forest fuel beds (Varner et al. 2015; Alexander et al. 2021), yet we do not fully understand if these traits remain consistent within a species across environmental gradients and/or disturbance histories.

Litter traits can affect any or all aspects of flammability and to different degrees. Based on a meta-analysis of seven different studies, leaf curling, surface area to volume (SA:V) ratio, and specific leaf area (SLA) were found to be more strongly correlated with flammability than leaf thickness, length, area, and tissue density (Burton et al. 2021). Leaf curling, defined as the maximum height of the leaf when laid horizontally on a flat surface without flattening and under laboratory, air-dried conditions (McDaniel et al. 2021), highly influences fuel bed structure, with flat leaves creating less aerated fuel beds, which in turn decrease fire rate of spread and flame height (Engber and Varner 2012; Varner et al. 2015; Grootemaat et al. 2017), and vice versa for

more curly leaves (Scarff and Westoby 2006; Engber and Varner 2012; Della Rocca et al. 2017). Among morphological litter traits, SA:V ratio was found to be the most crucial to flammability, with higher SA:V relating to shorter time-to-ignition and faster fire spread (Anderson 1970; Chandler et al. 1983; Dimitrakopoulos and Panov 2001; Weise et al. 2005; Saura-Mas et al. 2010; Simeoni et al. 2012; Engber and Varner 2012), as well as higher bulk density and higher fuel moisture (Rothermel 1972; Kreye et al. 2013; Babl et al. 2020). Leaf area also influences flammability, with smaller leaves leading to less aerated fuel beds, longer time to ignition, and lower rate of spread than larger leaves (Scarff and Westoby 2006; Murray et al. 2013; Grootemaat et al. 2015, 2017). Broader and wider leaves tend to ignite faster (Murray et al. 2013; Ganteaume 2018), and larger leaf area correlates with higher rate of spread, higher combustibility, and lower sustainability (de Magalhaes and Schwilk 2012; Krix et al. 2019). Thin leaves tend to burn with lower maximum temperatures, and leaf thickness negatively correlates with initial moisture content of litter beds, with thicker leaves having lower initial moisture content (Kreye et al. 2013; Grootemaat et al. 2017). Leaf thickness also correlates with longer time-to-ignition but a positive impact on sustainability (Grootemaat et al. 2017; Ganteaume 2018; Romero et al. 2019). Lower litter bulk density is associated with higher flammability due to its indication of higher heat absorption ability (Simeoni et al. 2012). All of these traits are important to our understanding of species flammability, and they tend to interact in ways that further change both species and overall forest flammability.

Individual leaf litter traits and individual species traits are important, but understanding how those traits and species interact with each other is another major focus of flammability research. As several studies have shown, species composition is a critical factor in flammability and fire behavior in mixed forests (de Magalhaes and Schwilk 2012; Zhao et al. 2016; Wyse et

al. 2018). Wang et al. (2009) developed a plant flammability prediction model that uses correlated species trait-based flammability variables, suggesting that the values of certain traits can be predicted from other known trait values. Individual traits have different importance to species flammability, especially as responses to its local environment, and this makes species flammability research difficult across larger areas (Kauf et al. 2015). We cannot evaluate the impact of a single trait on the fire behavior of a species without considering the influence of other traits (Schwilk 2015; Tumino et al. 2019). Interactions of species leaf litter traits can produce either synergistic or non-synergistic effects on flammability. For example, SLA and bulk density produce a synergistic effect on flammability, while high moisture content and large leaf area affect ignitability in opposite ways (Tumino et al. 2019). The combined effect of species-specific traits that either enhance or suppress flammability is even more complex in mixed forests because the species coexist in a way that expresses overall flammability through non-additive traits and different fire responses among species (de Magalhaes and Schwilk 2012; Van Altena et al. 2012; Zhao et al. 2016, 2019; Della Rocca et al. 2018; Wyse et al. 2018). Interactions of leaf litter traits are integral to forest flammability, and there is still much to be learned about variation in leaf litter traits, especially in the contexts of fire frequency and climate.

Fire and climate play an important role in the expression of plant traits and flammability, but there is little data about intraspecific variation and phenotypic plasticity in leaf litter traits due to these factors. Trait-based flammability studies have shown that fire-suppressing traits have an evolutionary role in forests, as exhibited by the wide range of flammability and trait differences across species groups, and that trait-based flammability is important for understanding the impact of historic and prehistoric fires (Cornwell et al. 2015; Belcher 2016).

Forest flammability is expressed at many levels, from organ to community, and fire influences adaptations, trait variation, and persistence of species in ecosystems with frequent fire (Pausas and Moreira 2012). The traits that influence flammability are typically hereditary, but they can be impacted by environmental factors, tree size, forest succession, and disturbance history (Rowe and Speck 2005; Babl et al. 2020; Kane et al. 2022). Fire behavior and flammability of plants can be influenced by these trait responses to environmental conditions (Blackhall et al. 2012; Jolly et al. 2012, 2016; Page et al. 2012, 2014; Krix and Murray 2018; Della Rocca et al. 2020; Michelaki et al. 2020). While we know that fire and environmental conditions can affect plant traits and flammability, we do not yet know how species leaf litter trait expression varies in burned and unburned areas or across a regional/climatic gradient.

Upland forests of the eastern and central United States are historically fire-dependent systems, but decades of fire suppression and other interacting factors have contributed to changes in forest flammability and encroachment of fire-sensitive, shade-tolerant species (Nowacki and Abrams 2008; McEwan et al. 2011; Hanberry et al. 2020; Alexander et al. 2021). The hypothesized process by which forest flammability has shifted is often referred to as mesophication, a positive feedback cycle in which conditions increasingly improve for shade-tolerant, fire-sensitive species and worsen for shade-intolerant, fire-tolerant species (Nowacki and Abrams 2008; Hanberry et al. 2014; Hanberry and Nowacki 2016; Alexander et al. 2021). This occurs through the increasing prevalence of species with traits known to reduce forest flammability and impede the growth and survival of fire-tolerant species. Hypothesized mesophytes (e.g., red maple/*Acer rubrum* L., sweetgum/*Liquidambar styraciflua* L., and tulip poplar/*Liriodendron tulipifera* L.) are species that are thought to contribute to mesophication through their canopy, bark, and leaf litter traits. For example, these species typically have wider,

deeper canopies that produce cooler and more shaded understory conditions and fuels that have higher moisture content, slower drying rates, and faster decomposition rates (Kane et al. 2008; Kreye et al. 2013; Babl et al. 2020; McDaniel et al. 2021; Alexander et al. 2021; Babl-Plauche et al. 2022). Pyrophytic species (e.g., many oaks/*Quercus* spp. L., pines/*Pinus* spp. L., and hickories/*Carya* spp. Nutt.) are those considered to be fire-adapted, many of which are disturbance-dependent and associated with a history of low-intensity surface fires (Abrams 2002; Kane et al. 2008, 2021; Gill et al. 2009; Ballard et al. 2017; Knapp et al. 2021; Varner et al. 2021, 2022). For instance, many upland oak species have adaptations that help them be more fire-tolerant, such as moderate shade-tolerance, deep root systems, resprouting abilities, and hypogeal germination (Abrams 2003; Brose et al. 2006; Royse et al. 2010; Arthur et al. 2012; Johnson et al. 2019). Pines and hickories often have highly flammable leaf litter as well (Greenberg and Collins 2021; Varner et al. 2021, 2022). Upland mixed forests and the changes they are undergoing make an excellent setting for the study of leaf litter traits and their influence on flammability.

The goal of this research is to better understand the drivers of forest flammability through investigation of leaf litter traits associated with flammability and comparison of traits at both the interspecific and intraspecific scales in upland forests of the eastern United States. Specifically, we want to know how species we typically consider to be pyrophytic or mesophytic compare in litter traits and whether there is variation in trait expression within species sampled from areas with different climatic or prescribed fire conditions. The specific objective of this study is to compare leaf litter traits associated with flammability across a gradient of species considered to be pyrophytic to mesophytic and intraspecific variation of species leaf litter traits across a regional climatic gradient and from both burned and unburned areas.

We hypothesize that species considered to be pyrophytic (e.g., *Quercus* spp. and *Carya* spp.) will exhibit greater values of leaf curling, leaf thickness, and leaf perimeter and lower values of bulk density, SA:V ratio and SLA than species considered to be more mesophytic (e.g., *Acer rubrum*, *Liquidambar styraciflua*, and *Liriodendron tulipifera*). The opposite is expected of the species considered to be more mesophytic, since lower values for these traits are associated with lower flammability. Pine (*Pinus* spp.) species are also expected to exhibit traits more closely aligned with higher flammability. Within the individual species, we hypothesize that litter trait variation will be more pronounced in litter sampled from warmer and drier climates than in cooler and more moist climates (Kane et al. 2022). Within individual species, litter sampled from unburned areas will express traits more consistent with decreased flammability (since fire suppression begets fire suppression), and litter sampled from burned areas will express traits more consistent with increased flammability. Furthermore, intraspecific variation in leaf litter traits will be more pronounced in litter collected from burned areas compared to litter collected from unburned areas, and variation in traits will likely be more due to fire history than due to climate factors (Anderegg 2022). Intraspecific variation in leaf litter traits will likely also vary within the species functional groups (i.e., oak pyrophytes, pine pyrophytes, and mesophytes) due to potential for local adaptation and species differences in fire-adaptive or non-fire-adaptive strategies (Kane et al. 2022). Ultimately, these results will help land managers better understand the variation in leaf litter fuel beds and how this variation may impact the flammability of stands where they want to return prescribed fire management.

Methods

Study Areas

Our study areas for the leaf litter collection included 12 locations across the southeastern United States: Bankhead National Forest, Bernheim Arboretum, Escambia Experimental Forest, Jones Center at Ichauway, Mary Olive-Thomas Demonstration Forest, Penny's Bend Nature Preserve, Spirit Hill Farm, Strawberry Plains Audubon Center, Tall Timbers Research Station, Talladega National Forest, Tallahatchie Experimental Forest, and Tuskegee National Forest (Figure 10). These sites occurred across a climatological gradient of annual precipitation (125.73 – 165.84 cm) and annual average temperatures (12.5 – 20.2 °C). The study areas represented a variety of forest types, including pine, hardwood, and mixed stands, and contained stands that experienced a variety of prescribed fire frequencies over the past decade. More detailed site information can be found in Table 3.

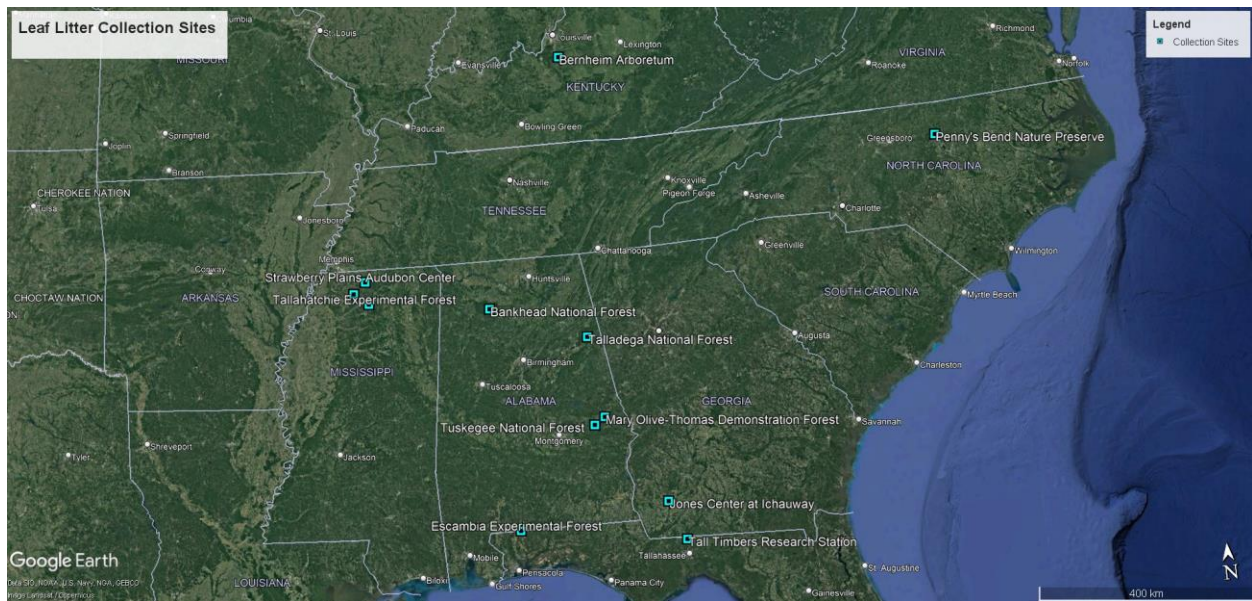


Figure 10. Map of the twelve leaf litter collection locations.

Table 3. Litter collection site information, including location, annual precipitation (cm), annual average temperature (°C), forest type, and prescribed fire frequency. All sites are in the U.S.A. Climate information from the National Oceanic and Atmospheric Administration National Centers for Environmental Information's U.S. Climate Normals data.

Site	Location	Annual Precipitation (cm)	Annual Average Temperature (°C)	Forest Type	Prescribed Fire Frequency
Bankhead National Forest	Double Springs, Alabama	153	15.7	Hardwood, loblolly pine	Unburned, 3 years, 9 years
Bernheim Arboretum	Louisville, Kentucky	133	14	Hardwood	Unburned, 3-5 year intervals
Escambia Experimental Forest	Brewton, Alabama	166	19.6	Longleaf pine	Unburned, 3-4 years
Jones Center at Ichauway	Newton, Georgia	126	19.2	Longleaf pine	Unburned, 1-2 years
Mary Olive-Thomas Demonstration Forest	Auburn, Alabama	143	17.6	Loblolly pine, hardwood	Unburned, 1 year, 2 years, 3 years
Penny's Bend Nature Preserve	Durham, North Carolina	119	15.1	Mixed pine-hardwood	Unburned, 2-3 years
Spirit Hill Farm	Holly Springs, Mississippi	141	15.3	Hardwood, mixed pine-hardwood	Unburned, Burned 2x in last 5 years
Strawberry Plains Audubon Center	Holly Springs, Mississippi	141	15.3	Hardwood	Unburned, 2-3 years
Tall Timbers Research Station	Tallahassee, Florida	149	20.2	Shortleaf pine, longleaf pine	Unburned, 1 year, 2 years, 3 years, 4 years
Talladega National Forest	Anniston, Alabama	146	15.7	Longleaf pine, shortleaf pine, hardwood	Unburned, 3-4 years
Tallahatchie Experimental Forest	Abbeville, Mississippi	154	15.3	Hardwood, shortleaf pine	Unburned, ~5 years
Tuskegee National Forest	Tuskegee, Alabama	138	17.6	Pine	Unburned, 3 years

Litter Collection

In January – February 2022, we collected leaf litter from eight sites across the southeastern U.S. We collected leaf litter from the remaining three sites from January – March 2023. At each site, we located a stand that had been burned regularly in the past 10 years and one that had no regular prescribed fire implementation for at least 10 years. We collected one medium-sized paper grocery bag full of litter per species from each stand. The litter was from species representing a range from pyrophytic to mesophytic species, with southern red oak (*Quercus falcata* Michx.), scarlet oak (*Q. coccinea* Wangenh.), white oak (*Q. alba* L.), post oak (*Q. stellata* Wangenh.), black oak (*Q. velutina* Lindl.), longleaf pine (*Pinus palustris* Mill.), shortleaf pine (*P. echinata* Mill.), loblolly pine (*P. taeda* L.), and mockernut hickory (*Carya tomentosa* Nutt.) being more representative of pyrophytes and red maple (*Acer rubrum* L.), sweetgum (*Liquidambar styraciflua* L.), and tulip poplar (*Liriodendron tulipifera* L.) being more representative of mesophytes (Greenberg and Collins 2021; Varner et al. 2021). As many species as possible were sampled on each site. Species with the most overlap across sites were chosen, but not every species was available on every site (Table 4).

Table 4. Species of litter sampled from each site. An asterisk (*) means that the species was collected at the site, but it was not collected at all fire frequency levels there. Z means that sand post oak (*Quercus margarettae* Small) was collected at one site at the Jones Center where post oak was not available, and Florida maple (*Acer floridanum* hort.) was collected at the Jones Center where red maple was not available.

Site	<i>Pinus palustris</i>	<i>P. echinata</i>	<i>P. taeda</i>	<i>Quercus falcata</i>	<i>Q. coccinea</i>	<i>Q. alba</i>	<i>Q. stellata</i>	<i>Q. velutina</i>	<i>Carya tomentosa</i>	<i>Acer rubrum</i>	<i>Liquidambar styraciflua</i>	<i>Liriodendron tulipifera</i>
Bankhead National Forest (AL)		X	X	X	X	X	X*	X	X	X		X
Bernheim Arboretum (KY)					X	X			X	X		
Escambia Experimental Forest (AL)	X			X			X			X	X*	
Jones Center at Ichauway (GA)	X	X*		X		X*	Z		X*	Z		
Mary Olive-Thomas Demonstration Forest (AL)			X	X		X	X		X	X	X	X
Penny's Bend Nature Preserve (NC)		X	X	X	X	X	X	X	X		X	X
Spirit Hill Farm (MS)			X	X		X	X		X	X	X	X
Strawberry Plains Audubon Center (MS)			X	X	X	X	X	X	X	X	X	
Tall Timbers Research Station (FL)	X	X	X	X		X	X	X	X	X	X	X
Talladega National Forest (AL)	X	X	X	X	X	X	X		X	X	X	X
Tallahatchie Experimental Forest (MS)		X	X	X	X	X	X	X	X	X	X*	X
Tuskegee National Forest (AL)	X	X	X	X			X	X	X	X	X	X

Litter Processing

For each collected litter sample (species/location/burned or unburned status combination), we took one sample to measure bulk density and then measured individual leaf litter traits for 30 leaves per sample. We measured leaf curling, leaf thickness, leaf perimeter, leaf one-sided surface area, oven-dried SLA, leaf volume, SA:V ratio, and leaf dissection index (LDI) for each of the 30 leaves. For bulk density (g/m^3), we filled a container of known volume with whole dried leaf litter and measured how many grams of litter fit into the container. For leaf curling (cm), we measured the maximum height of the leaf when laid horizontally on a flat surface without flattening and under laboratory, air-dried conditions. For leaf thickness, we had different protocols for pine and non-pine species. For pine species, we measured the thickness of each needle at its midpoint with calipers to the nearest 0.1 mm and then averaged those values; for non-pine species, we measured the leaf thickness with calipers to the nearest 0.1 mm at the mid-vein and leaf-edge after the leaf was bisected perpendicular to the mid-vein and then averaged those values. Leaf perimeter (cm), leaf one-sided surface area (cm^2), and exposed leaf area (cm^2) were all measured by scanning each leaf using a flatbed scanner and using threshold-based pixel count measurement in ImageJ software (Abràmoff et al. 2004). For non-pine species, we scanned each leaf once before flattening for the exposed area and once after flattening for the leaf perimeter and one-sided surface area. For pine species, the needles were spread out for the scan, and the leaf one-sided surface area was also used as the exposed leaf area due to inability to get reasonable measurements from doing an initial scan like the non-pine species. Oven-dried SLA (cm^2/g) was calculated by dividing the leaf one-sided surface area by its oven-dry mass. Leaf volume (cm^3) was calculated by multiplying the leaf one-sided surface area by the leaf thickness. SA:V ratio was calculated by dividing the two-sided surface area of the leaf by its

volume. Leaf dissection index (LDI) was calculated as the ratio of the leaf perimeter to the square root of the leaf one-sided surface area.

Data Analysis

Due to the highly correlated nature of our leaf litter trait response variables, we analyzed the data using a principal components analysis (PCA) at the individual leaf level. We then used the first two principal components from the analysis to create models; these principal components explained a cumulative 79.7% of the variation in the data. These models used species, location, and burn status as well as their interactions as predictors of the principal components in a multivariable linear regression. Bulk density was analyzed separately as the response variable in a similar model because it was measured at the collection site level rather than the individual leaf level.

Results

Our leaf litter traits tended to be highly correlated with one another (Figure 11), so we conducted a PCA to analyze these response variables. The first principal component was primarily representative of leaf curl, leaf one-sided surface area, exposed leaf area, oven-dry mass, and leaf volume (Table 5), with individuals exhibiting higher values of these traits located more on the left side of the axis and individuals exhibiting lower values of these traits located more on the right side of the axis (Figure 12). This component explained 46.1% of the variation in the data. For the first principal component, we observed a significant three-way interaction between species, burn status, and location ($p < 0.0001$). This means that species variations in leaf curl, leaf one-sided surface area, exposed leaf area, oven-dry mass, and leaf volume all depend

on burn status and location. For example, burned black oak in Bankhead National Forest exhibits leaf litter traits associated with higher flammability along this axis than unburned red maple at Tall Timbers Research Station.

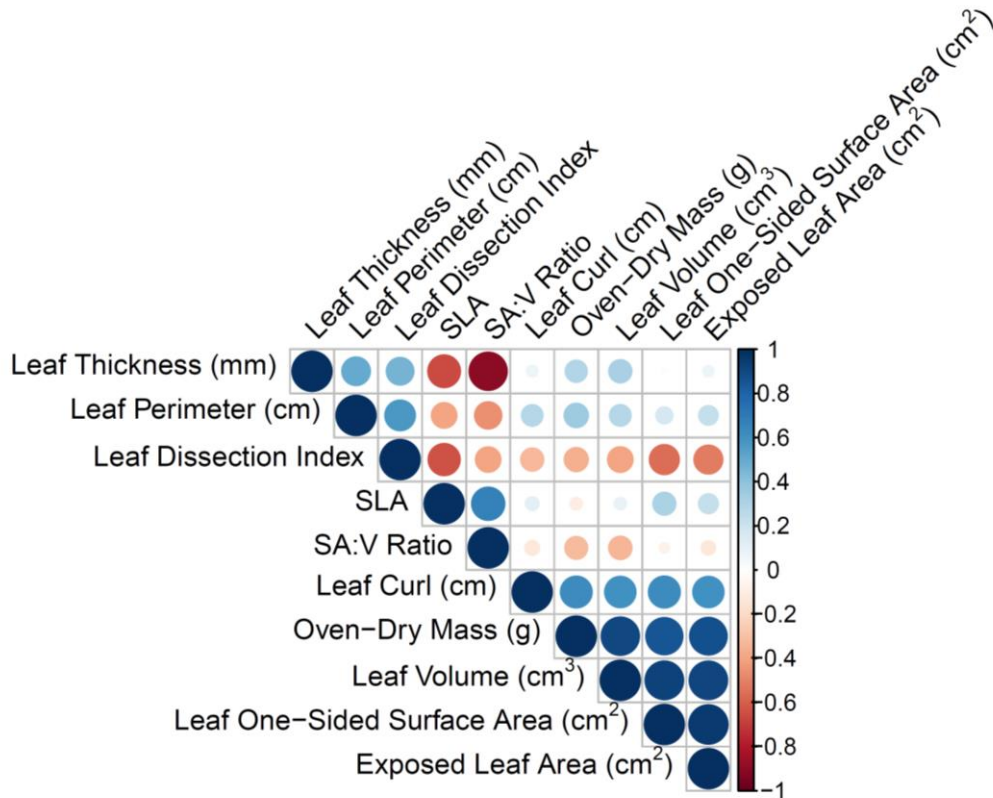


Figure 11. Correlation plot for measured leaf litter traits. Darker blue with larger circles represents stronger positive correlation, and darker red with larger circles represents stronger negative correlation between two traits.

Table 5. Principle component loadings for measured leaf litter traits in individual leaf data principal components analysis.

Litter Trait	PC1	PC2
Leaf Curl (cm)	-0.34	-0.02
Leaf Perimeter (cm)	-0.15	0.37
Leaf One-Sided Surface Area (cm ²)	-0.44	-0.12
Exposed Leaf Area (cm ²)	-0.44	-0.08
Oven-Dry Mass (g)	-0.44	0.07
Leaf Thickness (mm)	-0.11	0.48
SLA	-0.06	-0.47
Leaf Volume (cm ³)	-0.45	0.03
SA:V Ratio	0.13	-0.46
LDI	0.21	0.42

The second principal component was primarily representative of leaf perimeter, leaf thickness, SLA, SA:V ratio, and LDI (Table 5), with individuals exhibiting higher leaf perimeter, leaf thickness, and LDI located near the top of the axis and individuals exhibiting higher SLA and SA:V ratio located near the bottom of the axis (Figure 12). This component explained 33.6% of the variation in the data. For the second principal component, we observed a significant three-way interaction between species, burn status, and location ($p < 0.0001$). This means that species variations in leaf perimeter, leaf thickness, LDI, SLA, and SA:V ratio are dependent upon burn status and location. For example, burned black oak from Bankhead National Forest exhibited litter traits associated with higher flammability along this axis than unburned red maple from Tallahatchie Experimental Forest. For bulk density, we observed a significant interaction between species and location ($p = 0.007$).

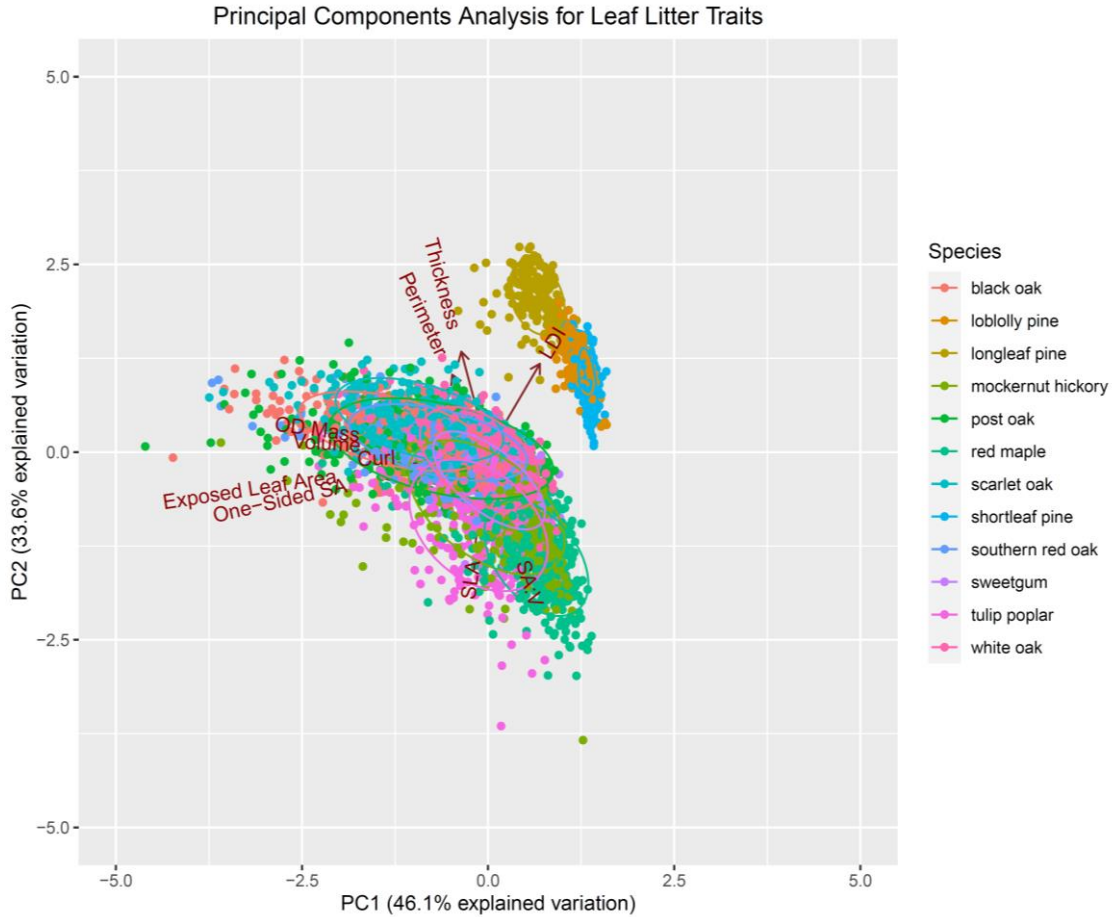


Figure 12. Principal components analysis plot using individual leaf litter trait data, grouped by species. Each point represents an individual leaf's measurements.

For the pine species, we observed intraspecific leaf litter trait trends with respect to burn status, location, and their interaction. Species PCA's broken down by burn status and location for loblolly pine, longleaf pine, and shortleaf pine are shown in Figure 13. For loblolly pine, we observed a significant interaction between burn status and location for both principal components ($p < 0.0001$ and $p = 0.04$, respectively). For longleaf pine, we observed only a significant burn effect ($p = 0.006$) for the first principal component, but the second principal component showed a significant interaction of location and burn status ($p < 0.0001$). For shortleaf pine, we observed a significant interaction of burn status and location for both principal components ($p < 0.0001$ and $p = 0.0008$, respectively). This means that intraspecific variation in leaf litter traits for

loblolly pine and shortleaf pine depend on both burn status and location, while intraspecific variation in leaf litter traits for longleaf pine depend only on burn status for some traits and on both burn status and location for others.

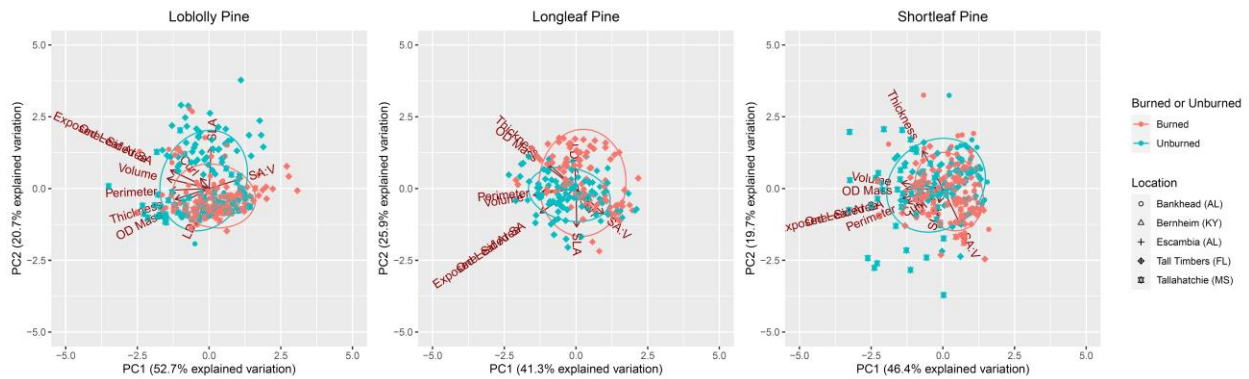


Figure 13. Principal components analysis plots for loblolly pine, longleaf pine, and shortleaf pine, using individual leaf litter trait data, grouped by burned or unburned status. Different shapes represent different collection locations. Each point represents an individual leaf.

For the pyrophytic oak species, we observed intraspecific leaf litter trait trends with respect to burn status, location, and their interaction. Species PCA's broken down by burn status and location for black oak, post oak, scarlet oak, southern red oak, and white oak are shown in Figure 14. For black oak, we observed a significant interaction of burn status and location ($p < 0.0001$) for the first principal component. The second principal component of black oak showed significant effects of burn status ($p = 0.009$) and location ($p < 0.0001$) but no significant interaction of burn status and location ($p > 0.05$). This means that within black oak, litter trait variation depends on the combination of burn status and location for some traits, but only on burn status and location individually for others. In post oak, we observed a significant interaction of burn status and location for both principal components (both $p < 0.0001$). For scarlet oak, we observed a significant interaction between burn status and location for both principal components

(both $p < 0.0001$). This means that for both post oak and scarlet oak, intraspecific leaf litter trait variation depends on the combination of burn status and location. For southern red oak, we observed a significant interaction between burn status and location for the first principal component ($p = 0.001$), but the second principal component showed only a significant location effect ($p < 0.0001$). This means that southern red oak leaf litter trait variation depends on the combination of location and burn status for traits such as leaf curl and leaf thickness, while only location influenced variation in traits such as LDI and SA:V ratio. For white oak, we observed only a significant effect of location ($p < 0.0001$) for the first principal component, but the second principal component showed a significant interaction of burn status and location ($p < 0.0001$). This means that only location influenced leaf litter traits such as leaf curl in white oak, but the combination of burn status and location influenced traits like LDI and SLA.

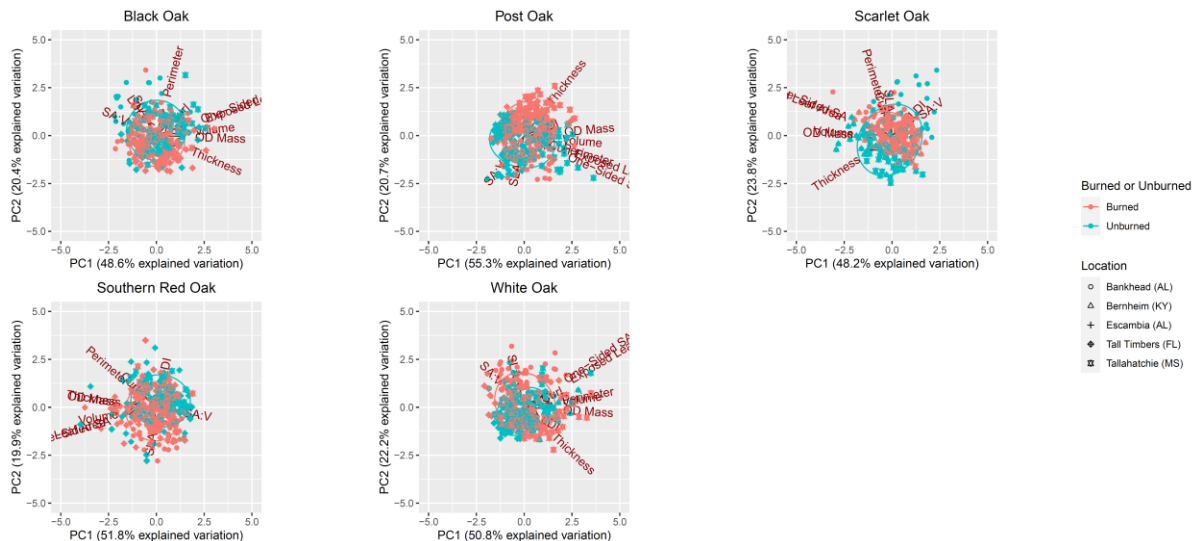


Figure 14. Principal components analysis plots for black oak, post oak, scarlet oak, southern red oak, and white oak, using individual leaf litter trait data, grouped by burned or unburned status. Different shapes represent different collection locations. Each point represents an individual leaf.

The other species also showed intraspecific leaf litter trait trends with regard to burn status, location, and their interaction. Species PCA's broken down by burn status and location for mockernut hickory, red maple, sweetgum, and tulip poplar are shown in Figure 15. For

mockernut hickory, we observed a significant interaction of burn status and location for both principal components ($p = 0.01$ and $p < 0.0001$, respectively). For red maple, we observed a significant interaction between burn status and location for both principal components (both $p < 0.0001$). This means that intraspecific litter trait variation for both mockernut hickory and red maple depends on the combination of burn status and location. For sweetgum, we observed only a significant effect of location for both the first principal component ($p = 0.0002$) and the second principal component ($p < 0.0001$), meaning that only location influences intraspecific trait variation for sweetgum. For tulip poplar, we observed significant effects for burn status ($p < 0.0001$) and location ($p < 0.0001$) individually but not for their interaction for the first principal component. The second principal component showed a significant interaction between burn status and location ($p < 0.0001$). This means that traits such as leaf curl and leaf thickness were influenced by burn status and location individually for tulip poplar, while intraspecific variation in traits such as LDI and SLA depended on the combination of burn status and location.

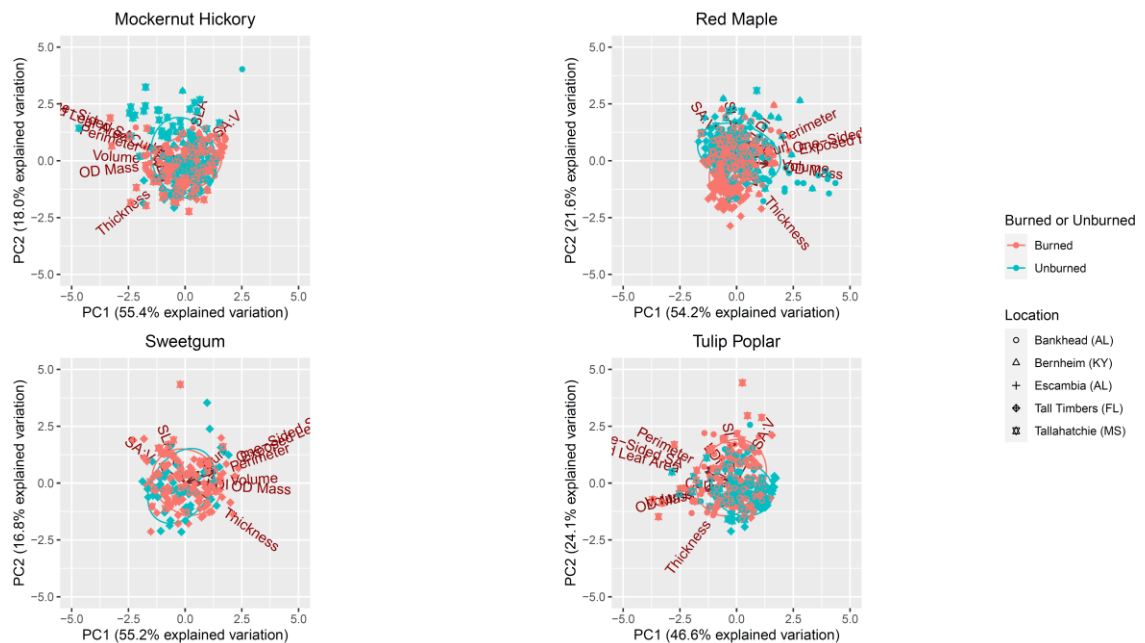


Figure 15. Principal components analysis plots for mockernut hickory, red maple, sweetgum, and tulip poplar, using individual leaf litter trait data, grouped by burned or unburned status. Different shapes represent different collection locations. Each point represents an individual leaf.

Discussion

In our study, we found that leaf litter traits associated with flammability have a wide range of interspecific variability, as commonly shown in the literature (Engber and Varner 2012; Varner et al. 2015; Popović et al. 2021), and intraspecific variability. Within species, we found that trait expression was influenced by burn status, location, and/or their interaction, depending on the species. This expansion of leaf litter trait knowledge will help managers better understand the flammability of stands with varying burn histories and species compositions, based on results from similar regions, and more effectively implement prescribed fire in areas that have been left unburned for long periods.

At the interspecific level, we observed a significant three-way interaction between species, burning, and location for both principal components. This suggests that variation in leaf litter traits depends on all three of these factors for our measured traits. Our results showed species forming two main groups: pine species and non-pine species. The pine species had their own gradient of leaf litter traits, from longleaf pine to shortleaf pine, and the non-pine species also formed a gradient of leaf litter traits, from more flammable black oak to less flammable red maple. Numerous previous studies have established that leaf litter traits such as these and the flammability associated with them differ between species (Engber and Varner 2012; Murray et al. 2013; Parsons et al. 2015; Popović et al. 2021), and our results support these findings, as well as showing a gradient of potential species flammability. It has also been proposed that functional traits such as these have weak relationships with climate and environmental factors (Anderegg 2022), but our results suggest that regional location, which we are using as a proxy for climatic factors, has an impact on differences in leaf litter traits at the interspecific level, especially when location interacts with species and/or burn status. Previous studies have suggested the idea that

flammability may be more environmentally determined than an adaptation in response to fire (Midgley 2013); however, we have observed that litter traits are also influenced by burn status. Analysis using more continuous predictor variables such as average annual temperature, annual precipitation, and latitude may result in conclusions that vary from our current analysis. While interspecific differences in leaf litter traits have been widely studied, data regarding the effects of burning and climate on intraspecific leaf litter trait variation are lacking in the literature.

Influences of burning, location, and their interaction on intraspecific leaf litter trait variation varied among the species we studied and even within species groups. We found a significant interaction between burning and location for many of the species, suggesting that, within a species, variation in leaf litter traits associated with flammability may depend on the combination of burning and location. We expected that intraspecific variation in litter traits would also vary within functional groups (i.e., oak pyrophytes, pine pyrophytes, and mesophytes) due to the potential for local adaptation and species differences in fire-adaptive or non-fire-adaptive strategies (Kane et al. 2022), and we observed this in how traits along each PCA axis responded differently to burning and location, even within species groups. In most cases, litter from burned sites exhibited traits that were consistent with higher flammability than litter from unburned sites, as we expected. Previous studies have found that flammability can be influenced by the presence or absence of fire, fire frequency, and the influence that it exerts on the traits of a species (Pausas and Moreira 2012; Cornwell et al. 2015; Belcher 2016). Our results suggest that fire exerts influence over intraspecific variation of leaf litter traits, but, in many species, the effect of burning was also dependent on the effect of location.

The influence of location on intraspecific litter trait variation was not clearly defined as if flammability had varied along a north-south gradient by locations like we might have expected.

Some intraspecific studies have been conducted using more northern pine species, such as pitch pine (Kane et al. 2022), that suggest that regional location may impact intraspecific differences in pine leaf litter traits, as our results have also shown. Locations that were farther apart tended to have larger differences than locations that were closer to one another, but the location effect is dependent on the burn effect for the majority of the species. While we used location as a proxy for climatic variation, the observed differences in leaf litter trait variation that depend on location may be due to leaf litter trait responses to many factors: climatic or other environmental factors, hereditary population differences, tree size, forest succession, and disturbance history at these locations (Rowe and Speck 2005; Blackhall et al. 2012; Jolly et al. 2012, 2016; Page et al. 2012, 2014; Krix and Murray 2018; Della Rocca et al. 2020; Michelaki et al. 2020; Babl et al. 2020; Kane et al. 2022). As with the effect of burning, the influence of location on leaf litter traits was often intertwined with influence of fire for many species, making the individual effects of each factor difficult to discern.

While the nuances of forest flammability, especially in mixed forests, are difficult to simplify, our findings have both basic science and management implications. Understanding how intraspecific variation in leaf litter traits associated with flammability is influenced by burning, location, and their interaction is a key step in unravelling the tangled web of forest flammability. In systems undergoing mesophication (Nowacki and Abrams 2008), like the ones where we collected litter for this study, returning fire to the system is a complex process that requires more than just typical prescribed fire and management practices (Alexander et al. 2021; Cabrera et al. 2023). Factors such as species composition (de Magalhaes and Schwilk 2012; Zhao et al. 2016; Wyse et al. 2018), relative importance of traits (Kauf et al. 2015), interactions of traits (Schwilk 2015; Tumino et al. 2019), and mixed forest flammability dynamics (de Magalhaes and Schwilk

2012; Van Altena et al. 2012; Zhao et al. 2016, 2019; Della Rocca et al. 2018; Wyse et al. 2018) all make understanding forest flammability even more difficult and enigmatic. Gaining better insight into how intraspecific variation in leaf litter traits responds to burning and location will allow more informed efforts for returning fire to systems undergoing the mesophication process. For example, if we observe that a burned post oak from Tallahatchie Experimental Forest exhibits traits associated more with high flammability than an unburned post oak from the same location, then the fire behavior of post oak in the unburned stand could be shifted from the typical flammability of the species. This, in turn, could reduce the overall flammability of the stand and require a different approach (e.g., burning under different conditions or in a different season) to effectively return fire to the unburned stand. Another example is if a burned red maple exhibits traits more aligned with higher flammability at a location, then, over time, returning fire to an unburned stand may lead to increased flammability in even the typically less flammable species.

Conclusions

Leaf litter traits associated with flammability vary widely in their expression based on species differences and intraspecific variation correlated with regional location and management by prescribed fire. Understanding the nuances of these differences will provide better understanding of forest flammability and allow managers to use this knowledge for better predictions of how fire may behave in a stand that is in a similar region with a similar species composition and fire history, and ultimately provide insight into how to effectively return fire to long unburned forest systems. Further research is needed to expand the current leaf litter trait

datasets to cover more species over a larger regional spread and wider range of prescribed fire frequencies.

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