Chinese tallow seed viability, resprouting capacity, and leaf litter flammability: consequences for restoring coastal pine forests

by

Eric Scott Brubaker

A thesis submitted to the Graduate Faculty of
Auburn University
in partial fulfillment of the
requirements for the Degree of
Master of Science

Auburn, Alabama
August 5, 2023

Keywords: invasive, prescribed fire,
sprouting, *Triadica sebifera*

Approved by

Heather D. Alexander, Co-chair, Associate Professor of Forest Ecology, College of Forestry, Wildlife, and Environment
Joseph Fan, Co-chair, Professor of Forest Ecology and Statistics, College of Forestry, Wildlife, and Environment
Christopher J. Anderson, Professor of Wetland Ecology, College of Forestry, Wildlife, and Environment

Abstract

Chinese tallow (*Triadica sebifera*) is an invasive tree that threatens the health and integrity of pine flatwoods and savannas along the USA Gulf of Mexico. These open forests historically experienced frequent, low-intensity surface fires, but tallow invasion likely threatens fire potential through ecosystem transformation. Here, we sought to better understand the mechanisms tallow uses to survive and proliferate in these threatened ecosystems by exploring three components of tallow invasion: seed viability, resprouting capacity, and litter flammability. In general, tallow seed viability declined rapidly after dispersal, and seedling establishment rates were similarly low. Tallow leaf litter drastically reduced fire ignition, rate of spread, and fuel consumption, a potential mechanism that could promote further tallow invasion in the absence of fire and drive ecosystem transformation. Although tallow can vigorously resprout, tree size, canopy closure, and soil EC reduced tallow survival following top-kill and subsequent resprouting.

Acknowledgements

I extend my gratitude to my major professors, Drs. Heather Alexander and Joseph Fan, for their support and guidance in developing this research project. I thank my committee member Dr. Chris Anderson for his feedback, support, and contribution to this project's experimental design. Assistance from current and former graduate and undergraduate members of Auburn University's Forest and Fire Ecology Lab (Emily Acer, Payton Brewer, Eric Bridges, Steven Cabrera, River Collins, Hudson Defee, Kathleen Gabler, Lucas Hart, Max Heaven, Luiza Goncalves Lazzaro, Hannah Manire, Katelyn McBride, Patrick McMahon, Alice Martineau, Tamara Milton, Arthur Lamounier Moura, Rachel Nation, Dylan Ogle, and Michael Ramirez) and the Natural Resource Biometrics Lab (Haomin Haung, Favour Onyido, and Shaoyang Yang) as well as undergraduate volunteers (Brandon Barnett and Brianna Travis) made fieldwork and lab work for this project possible. I also thank Drs. Carol Baskin, Ian J. Renne, Todd Steury, and John Willis for their assistance with experimental design, data analysis, and sample processing. Lastly, I thank the staff of Grand Bay National Estuarine Research Reserve and Mississippi Sandhill Crane National Wildlife Refuge for their assistance with this project and support in allowing us access to these study areas. This research project was funded by NIFA Hatch Projects (#ALA031-1-19129 and #ALA0:031-1-17108), NIFA McIntire-Stennis Projects (#ALAZ-00079 and #ALAZ-00075), the cooperative grant (FS-22-CS-11330170-055) from the US Forest Service Southern Research Station, and a JFSP GRIN grant (BLM-L23AC00041-00).

Table of Contents

List of Tables

List of Figures

List of Abbreviations

ANOVA	analysis of variance
DBH	diameter at breast height
EC	soil electric conductivity (mS/cm)
GB	Grand Bay National Estuarine Research Reserve
GOM	Gulf of Mexico
MOT	Mary Olive Thomas Demonstration Forest
NERR	National Estuarine Research Reserve
NWR	National Wildlife Refuge
SSD	scarified seed(s)
SH	Mississippi Sandhill Crane NWR
tNSC	total nonstructural carbohydrates
TZ	tetrazolium chloride
USA	United States of America
USD	unscarified seed(s)

Chapter 1: Chinese tallow (*Triadica sebifera*) seed viability and early seedling establishment in fire-dependent slash and longleaf pine (*Pinus elliottii; P. palustris*) savannas and flatwoods along the Gulf of Mexico Coast

Abstract

In the southern USA, endangered longleaf and slash pine (*Pinus palustris*, *P. elliottii*) flatwoods are at risk of invasion from Chinese tallow (*Triadica sebifera*; hereafter: tallow) – an ecosystem transforming invasive exotic tree species with prolific seed production. This study had two primary aims: to assess changes in tallow seed viability over time and at different depths in the soil seedbank and determine the effect different fuel complexes have on early seedling establishment. To assess changes in seed viability over time and at different soil depths between seeds dispersed by different mechanisms, hydrochory (water dispersal; unscarified) and zoochory (animal dispersal, specifically by birds; scarified), we established a total of 30 plots at Mississippi Sandhill Crane National Wildlife Refuge, Mississippi, USA in May 2022. Each plot contained bags with seeds from two scarification treatments (Scarified, SD, and Unscarified, USD) placed at three depths at or below the soil surface (0, 2, and 5 cm) that were collected 3-, 6-, 9-, and 12-months post planting. Seed viability was determined using the tetrazolium (TZ) test by allowing seed embryos to imbibe in a 1% TZ solution for three days, and seeds stained red were presumed viable. Prior to planting, baseline viability was 92% and 94% for SD and USD seeds, respectively. Viability decreased rapidly after planting; however, USD seeds that still had the waxy coat had a slower decline in viability than SD seeds, suggesting an interaction between scarification treatment and time. 3- and 6-month viability for USD and SD seeds was 21% and 6%, and 13% and 5%, respectively. Seed viability was similar between both scarification treatments at ~5% at 9- and 12-month collections times. Regardless of time post-

planting or scarification treatment, depth below the soil surface did not affect the viability of seeds. These results demonstrate a sharp initial decrease in tallow seed viability regardless of dispersal mechanism or depth below the soil surface which could limit the ability of tallow seeds to persist long-term in the soil seedbank. To assess differences in seedling establishment over time between different fuel complexes in both burned and unburned sites, we established 30 plots with 100 tallow seeds each in five different fuel complexes at our Mississippi study site in April 2022: native grass (NG), mixed grass/shrub (GS), mixed pine (MP), pine only (PO), and cogongrass (CG), and recorded early seedling establishment for a one-year period. We found no significant difference in seedling density between native fuel complexes; however, seedling density one-year post-planting was ~2.0 times higher in unburned than burned sites. These results demonstrate tallow seed's ability to establish in the soil seedbank or become established seedlings is low, suggesting that the removal of mature, seed producing trees should be a primary objective in invaded ecosystems.

Introduction

Coastal longleaf and slash pine (*Pinus palustris* and *P. elliottii*, respectively) savannas and flatwoods of the southeastern United States require frequent low-intensity surface fires with an annual to 3-year fire return interval to maintain their composition, structure, and biodiversity, and to provide favorable habitat conditions for numerous wildlife species including the federally endangered Mississippi sandhill crane (*Grus canadensis pulla*). As such, prescribed fire is a commonly used management tool to preserve the integrity of these pyrophytic ecosystems (U.S. Fish and Wildlife Service, 2010). However, this practice can also create favorable conditions for the establishment and proliferation of invasive plant species, thereby threatening the utility of prescribed fire efforts (Yang et al., 2019).

Along the Gulf of Mexico coast, one invasive plant species of concern in fire-dependent forest ecosystems is the Chinese tallow tree (*Triadica sebifera* (L.) Small.; hereafter: tallow), an invasive exotic expected to expand its range throughout the southeastern USA due to climate change, prolific seed production (Grace, 2001; Lin et al., 1958; Renne et al., 2002; Wang et al, 2011), and long-distance seed dispersal by water and birds (Renne et al., 2001; 2002). Tallow is an ecosystem transformer that creates a dense midstory which increases soil moisture and shades out native, often herbaceous, understory species (Grace, 1998). Coastal pine savannas and flatwoods provide ideal canopy structures for bird perches, likely facilitating tallow dispersal in these systems (Fan et al., 2021a). Due to tallow established in these threatened ecosystems, it is critical to understand the interactions between natural processes such as fire and tallow spread and proliferation.

Tallow seeds have a thick, waxy seedcoat to aid seed dispersal by water (hydrochory) (Renne et al., 2001; Cameron & Spencer, 1989), which could impact seed viability in both unburned and burned environments. Tallow seeds distributed by birds (zoochory) differ from those distributed by water in that they have the seedcoat removed via scarification as the seed passes through a bird's digestive tract (Renne et al., 2002; Pile et al., 2017) and subsequently germinate at higher rates than unscarified seeds (Renne et al., 2001). However, seedcoats play an important role in protecting seeds from both physical stress and microorganisms (Mohamed-Yasseen et al., 1994). Since tallow seeds can readily establish in the soil seedbank for up to five years up to 5 cm below the soil surface (Cameron et al., 2000; 2004; Fan et al., 2021a), it is important to understand how the two primary mechanisms of dispersal, zoochory and hydrochory, differ in viability over time. Additionally, the vertical distribution of seeds in the soil seedbank may alter the viability of tallow seeds over time. Many weed species see significant declines in viability as depth below the soil surface increases (Conn et al., 2006; Mennan & Zandstra, 2006), and seedling emergence for seeds deeper in the soil profile is lower than for seeds near the soil surface (Zhang et al., 2019). The combined effect of depth below the soil surface and dispersal mechanism could play an important role in how long tallow seeds can remain viable in at-risk ecosystems.

Fire could have either negative or positive impacts on tallow seedling establishment in areas that maintain their capacity to burn. Tallow seeds do not require scarification to germinate; however, seeds scarified by passing through a bird's digestive tract have a higher likelihood of germinating (Renne et al., 2001). Because fire can scarify seeds of other invasive species (Susko et al., 2011), fire may do the same for tallow seeds. Notably, tallow seedling establishment increases post burn which suggests that fire could scarify the seeds of this species and prime the soil surface for seedling emergence (Fan et al., 2021b). Additionally, temperature fluctuation resulting from a fire could also promote tallow germinant emergence (Nijjer et al., 2002).

However, fire temperature may influence whether seeds survive a burn or not. Less severe fires that burn only surface fuels could act as a scarification mechanism, promoting seed germination, while more severe fires could kill the tallow seeds. Different fuel types and canopy structures can alter fire temperature (Groeschl et al., 1993, Grace, 1998); therefore, tallow seeds in different fuel types may have different seedling establishment rates following a burn.

For this study, we had two primary objectives exploring tallow seed viability and early seedling establishment in fire-dependent forests along the Gulf of Mexico coast. The first objective of this study was to determine the effects time in soil, dispersal method, and position in the soil profile have on the viability of tallow seeds. We hypothesized that tallow seeds on the soil surface would suffer damage from frequent fires and harsh environmental conditions, resulting in lower viability. Seeds 2-cm below the soil surface will be shielded from prescribed fire damage, allowing them to survive and have the highest viability. Seeds at the deepest position 5-cm below the soil surface will imbibe with water due to higher soil moisture levels leading to similarly low viability to the surface seeds. Across all depths below the soil surface, tallow seed viability will decrease as the time in soil increases, but seeds distributed via water with their waxy coat intact will have higher viability compared to those distributed via birds that have been scarified.

The second objective was to determine the effect of common fuel types (1: native grasses, 2: mixed grass/inkberry (*Ilex glabra*), 3: pine (*Pinus* spp.) and native grass mix, and 4: pine litter, and 5: cogongrass (*Imperata cylindrica*)) present at Mississippi Sandhill Crane National Wildlife Refuge and Grand Bay National Estuarine Research Reserve have on early seedling establishment of tallow following prescribed fire. For this objective, we hypothesized that tallow seeds burned in plots with mixed grass/inkberry would have the highest germination rate due to the heterogeneity of fuels in this fuel type which could reduce maximum temperatures. Tallow seeds burned in native grasses and the pine and native grass mix fuel types will have an intermediate mortality rate and lower germination rates than the seeds in mixed grass/inkberry fuels due to increased fire temperature in these fuel types. Finally, seeds burned in cogongrass will have the highest mortality rate and lowest germination rate due to the high fire temperature this fuel type burns at.

Methods

Study Sites:

We conducted research at two study sites containing pyrophytic pine flatwoods and savannas in Jackson County, Mississippi, USA: 1) Mississippi Sandhill Crane National Wildlife Refuge (NWR) (30.475°N, 88.783°W) and 2) Grand Bay National Estuarine Research Reserve (NERR) (30.377°N, 88.434°W). Mississippi Sandhill Crane NWR receives 175 cm of rainfall annually, peaking between June and August, and has an average high and low temperature of 26 °C (July) and 12 °C (January), respectively. Grand Bay NERR has a similar climate with an annual mean of 169 cm of precipitation that peaks in July and average high and low temperatures of 26 °C (July) and 12 °C (January), respectively (NOAA, 2021). Both study sites fall within the Gulf Coastal Plain, but Grand Bay NERR is closer to the Gulf of Mexico Coast (< 2-km average) and has a lower average elevation (~ 2-m) than Mississippi Sandhill Crane NWR that sites approximately 9-km from the Gulf of Mexico coast and has a 9-m average elevation. Soils for both study sites are similar, however, Grand Bay NERR has a predominance of poorly drained silty loams and loamy sands while Mississippi Sandhill Crane NWR is characterized by very fine sandy loams and loamy alluvium (NRCS, 2021).

Flatwood and savanna forests found along the Gulf of Mexico coast with predominantly longleaf and slash pine (*Pinus palustris, P. elliottii*) canopies dominate both sites. Both forest types have an absent midstory and a rich, diverse ground layer of herbaceous vegetation including wiregrass (*Aristida stricta*), toothache grass (*Ctenium aromaticum*), and insectivorous plants (*Sarracenia* spp., *Pinguicula* spp., and *Drosera* spp.) as well as low shrubs such as inkberry (*Ilex glabra*) and gallberry (*Ilex coriacea*) (personal observations, 2021-2023). Fuels are primarily herbaceous plants and pine litter in both community types. Active management for the care of refuge wildlife species has been taking place since the late 1970s. This includes frequent (1- 3 year fire return interval) low-intensity prescribed fires typically applied between March and November to promote seeding of native grasses needed by endangered species that call the refuge home. Additionally, both study sites experience biological invasion from several undesirable species such as cogongrass (*Imperata cylindrica*), camphor tree (*Cinnamomum camphora*), and tallow, making them ideal candidates for our research.

Tallow Seed Viability

To determine how scarification, depth below the soil surface, and time in soil impact tallow seed viability, we outplanted seed bags filled with unscarified and scarified tallow seeds placed at three depths (surface, 2 cm, and 5 cm) and removed at different times since planting in 30 plots (n = 15/site) distributed across Mississippi Sandhill Crane NWR and Grand Bay NERR starting in May 2022. Plots were established randomly in pine flatwoods and savannas with a minimum spacing of 100 m between each. Each 2-m plot contained two 0.5-m subplots ~ 1-m apart and marked in the center with a 0.5-m piece of rebar to facilitate plot re-location and to secure seed bags (Figure 1-1).

Figure 1-1. Seed bag plot consisting of 13 seed bags (A) between two subplots with rebar stakes at their center (B) in Mississippi Sandhill Crane NWR and Grand Bay NERR, southern Mississippi, USA.

To construct seed bags, we collected tallow seeds by hand from six mature trees ranging in age from 12 – 26 years old and size from 13 – 30 cm DBH at Mississippi Sandhill Crane NWR in October 2021 and stored them in a dark, climate-controlled room (19.5 – 21 °C) until further processing.

In November 2021, we scarified a subsample of tallow seeds following the methods of Renne et al. (2001) to simulate the passage through a bird's digestive tract, as zoochory, specifically by birds, is the primary dispersal method for tallow seeds (Renne, 2001; 2002; Fan 2018; Fan et al., 2021). Briefly, the seeds were soaked in hydrochloric acid (38.5%) for 40 minutes, carefully removed from the acid, washed with water three times to remove any residual acid, and were allowed to dry overnight.

Scarified and unscarified seeds were then placed in fine mesh aluminum screening seed bags (10 cm x 8 cm), which were held together with metal staples and folded edges to contain the seeds and designed to resist damage from fire that frequently occurs at our study sites. Each bag contained 30 tallow seeds: 10 positioned at each of three depths (0, 2, and 5 cm) below the soil surface. At each depth, the seeds were divided by their scarification treatment: scarified and unscarified (Figure 1-2a). To distinguish between these the two treatments, a small, bright orange plastic ball was placed with the scarified seed treatment (Figure 1-2b).

In May 2022, we planted 13 seed bags (1 bag for each of 12 pick-up times plus one extra in case of damage) with a dibble bar at each plot to test how seed viability changes with time since planting. At each plot, six or seven bags were attached to each subplot rebar in a circular fashion using a 0.5-m piece of wire to ensure no seeds were left on site or lost. One seed bag was collected randomly from each plot at each pick-up date at the 3- (August 2022), 6- (November 2022), 9- (February 2023), and 12-month (May 2023) pick up dates. Future collections will take place 18-, 24-, 36-, 48-, 60-, 72-, 84-, and 96-months post planting to see how viability trends continue over time.

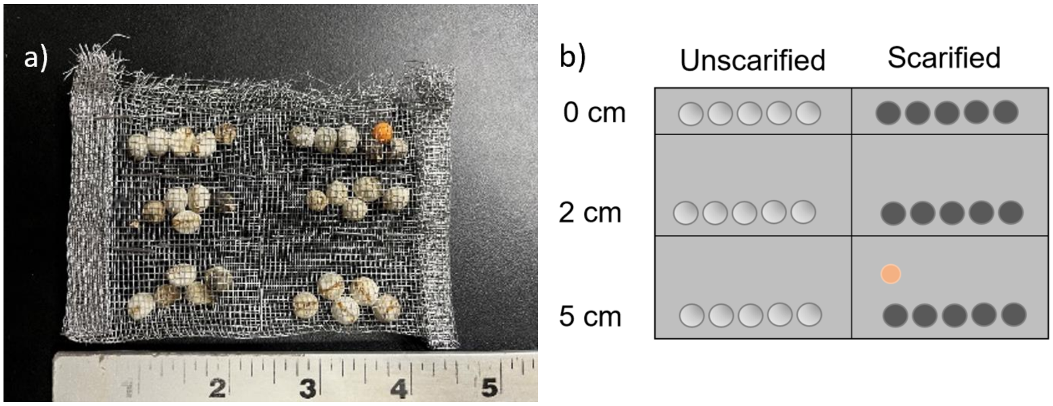

Figure 1-2. Seed bag (a) prior to outplanting at Mississippi Sandhill Crane National Wildlife Refuge (Ocean Springs, Mississippi, USA) and Grand Bay National Estuarine Research Reserve (Moss Point, Mississippi, USA) and (b) design concept showing tallow *(Triadica sebifera)* seeds with three depths below the soil surface (0, 2, and 5 cm) and two scarification treatments (scarified and unscarified) to be installed.

To determine viability, collected seeds were removed from the seed bag, placed in a 1% tetrazolium chloride (TZ) solution, and allowed to imbibe for 72 hours. Following soaking in the TZ solution, we dissected each seed to assess viability. Seeds stained red from the TZ solution were assumed viable, and seeds that were white or brown were presumed to be dead (Conn et al., 2006, Moore, 1985; Figure 1-3).

Figure 1-3. Viable (left, red) and dead (right, white) tallow (*Triadica sebifera*) seed embryos following 72 hours soaking in a 1% tetrazolium chloride (TZ) solution.

Seedling establishment

To determine the impact of different naturally occurring fuel complexes on tallow early seedling establishment, we identified units in our study site slated to be burned in 2022. Prior to burning in April 2022, we identified common fuel types that occur frequently throughout Mississippi Sandhill Crane NWR and Grand Bay NERR: 1) native grasses, 2) mixed native grasses and inkberry (*Ilex glabra*), 3) mixed pine (*Pinus* spp.) litter and native grasses, 4) pine litter, and 5) cogongrass (*Imperata cylindrica*). Within each identified fuel type, three 0.25-m^2 plots were established in homogonous patches of a given fuel type no closer than 10 m from each other. In April 2022, we clipped vegetation and collected litter from pine and hardwood trees to quantify fuel loading by functional group within our plots. These samples were placed in brown paper bags and transported back to Auburn University for additional processing. We sorted each fuel type into four categories: 1) grasses and herbs, 2) hardwood litter, 3) pine litter, and 4) shrub. Following sorting, these samples were dried at 60 ° C for 72 hours and subsequently weighed. After weighing, the percent grass, hardwood, pine, and shrub contribution to the fuelbed was calculated and averaged across the replicate plots for each fuel type.

To determine how fuel type and fire temperature influence early seedling establishment for tallow, we created three replicate 0.25-m^2 plots adjacent to the previously quantified five fuel types (n = 3 plots per fuel type per site, n = 2 sites, for a total of 30 plots). In each of these plots, 100 tallow seeds were systematically planted in a 2.5 x 2.5-cm grid in April 2022. In May 2022, after all 30 of the plots were established with tallow seeds, 15 plots in Mississippi Sandhill Crane NWR were burned. For this objective, we intended to have all our plots burned at our study sites. Unfortunately, one of the units in Grand Bay NERR we selected for this project did not get burned in the window we anticipated. As a result, half of our early seedling establishment plots remained unburned. However, we still monitored seedling establishment in these plots throughout the study period.

One month post burn, we checked for seedling emergence at all 30 plots (both burned and unburned), marked new seedlings with a pin flag, and recorded the total foliar cover as well as percent cover for grass, woody, leaf litter, and exposed mineral soil of each plot using a Daubenmire class scale (Daubenmire, 1959). Additional visits were made to the sites in November 2022, March 2023, and May 2023 to document new seedling emergence and mortality of previously recorded seedlings.

Data analysis

Using a linear mixed effects model, we tested for differences in seed viability and interactions by time at all collection points post planting (excluding initial viability), planting depth, and scarification treatment with study site location as a random effect in the model and a significance of $\alpha = 0.05$. Since the model indicated a significant interaction between scarification treatment and time post planting, we conducted pairwise comparisons to assess the effect size of this interaction at each collection time. For early seedling establishment, we used a linear mixed effects model to assess the effect of burning and fuel complex on seedling establishment with study site location as a random effect. Additionally, an analysis of variance (ANOVA) was performed to see if fuel complex alone impacted seed viability. We conducted all statistical analyses using R-4.2.1 (R Core Team 2021).

Results

Seed viability

Our model indicated an interaction between seed scarification treatment and time ($p < 0.001$), so we compared viability of seeds by scarification treatment at each collection time post planting. Prior to outplanting, tallow seed viability was not significantly different ($p = 0.29$) between scarified and unscarified seeds (92 and 94%, respectively). At 3-mo post planting, unscarified seed viability was 14.2% (+/- 0.044%, 95% CI) higher than scarified seeds ($R^2 = 0.19$, $p < 0.001$). Similarly, at 6-mo post planting, unscarified seeds had 8% (+/- 0.039, +/- 95% CI) higher viability than unscarifed ($R^2 = 0.09$, $p < 0.001$). Viability was not different between scarification treatments at 9-mo ($p = 0.474$) and 12-mo ($p = 0.999$) post planting (Figure 1-4). Regardless of scarification treatment or time post planting, depth below the soil surface had no significant impact on viability (Table 1-1).

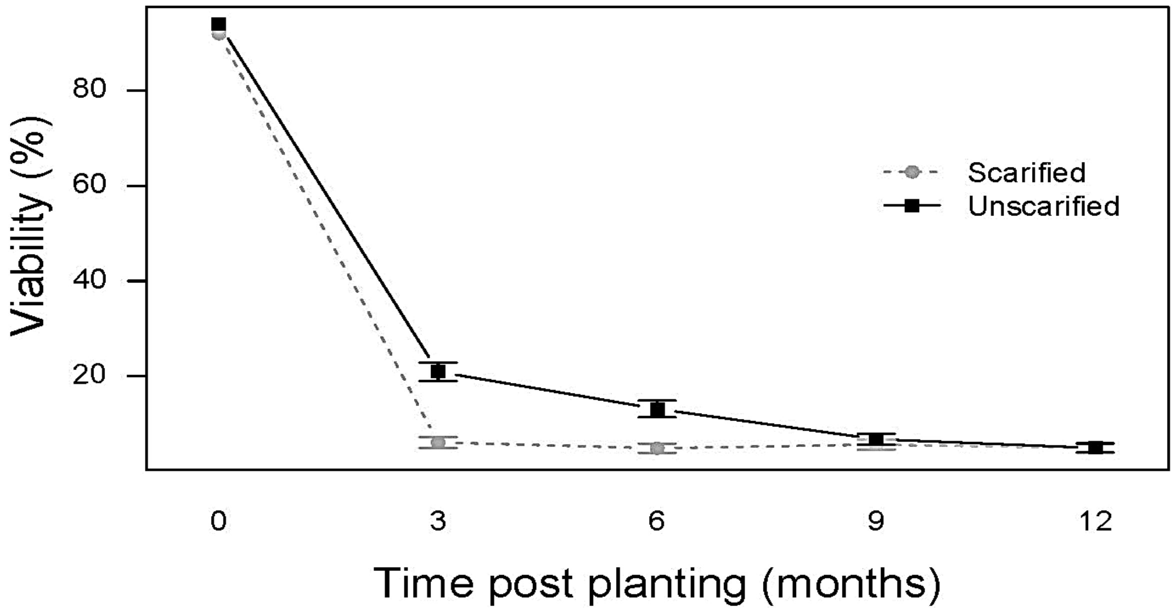

Figure 1-4. Line plot of interaction between scarification treatment and seed viability at each collection time from seeds outplanted in southern Mississippi, USA in May 2022.

Table 1-1. Mean (sd) seed viability by depth below the soil surface and scarification treatment (scarified, SCD; unscarified, USD) at 3-, 6-, 9-, and 12-months post planting from seeds planted in southern Mississippi, US.

	Time											
	3-mo			6-mo			9-mo			12-mo		
Depth	0 cm	2 cm	5 cm	0 cm	2 cm	5 cm	0 cm	2 cm	5 cm	0 cm	2 cm	5 cm
SCD	0.06	0.05	0.07	0.03	0.06	0.06	0.03	0.08	0.06	0.05	0.07	0.03
	(0.11)	(0.10)	(0.12)	(0.07)	(0.11)	(0.09)	(0.08)	(0.11)	(0.09)	(0.10)	(0.11)	(0.08)
USD	0.18	0.24	0.21	0.12	0.13	0.14	0.09	0.05	0.06	0.03	0.07	0.04
	(0.18)	(0.18)	(0.18)	(0.14)	(0.17)	(0.18)	(0.11)	(0.12)	(0.11)	(0.08)	(0.10)	(0.10)

Seedling establishment

Tallow early seedling establishment was 2.04 (1.35 – 3.13; 95% CL) times higher in unburned compared to burned plots ($p < 0.001$), and seedling establishment remained similar over time with no significant mortality or recruitment over the 1-year observation period ($p = 0.798$, Figure 1-5). ANOVA revealed no significant differences in early seedling establishment in burned plots by fuel complex ($p = 0.084$, Figure 1-6) despite differences in fuel loading (g/m^2), and burn temperature (Table 1-2).

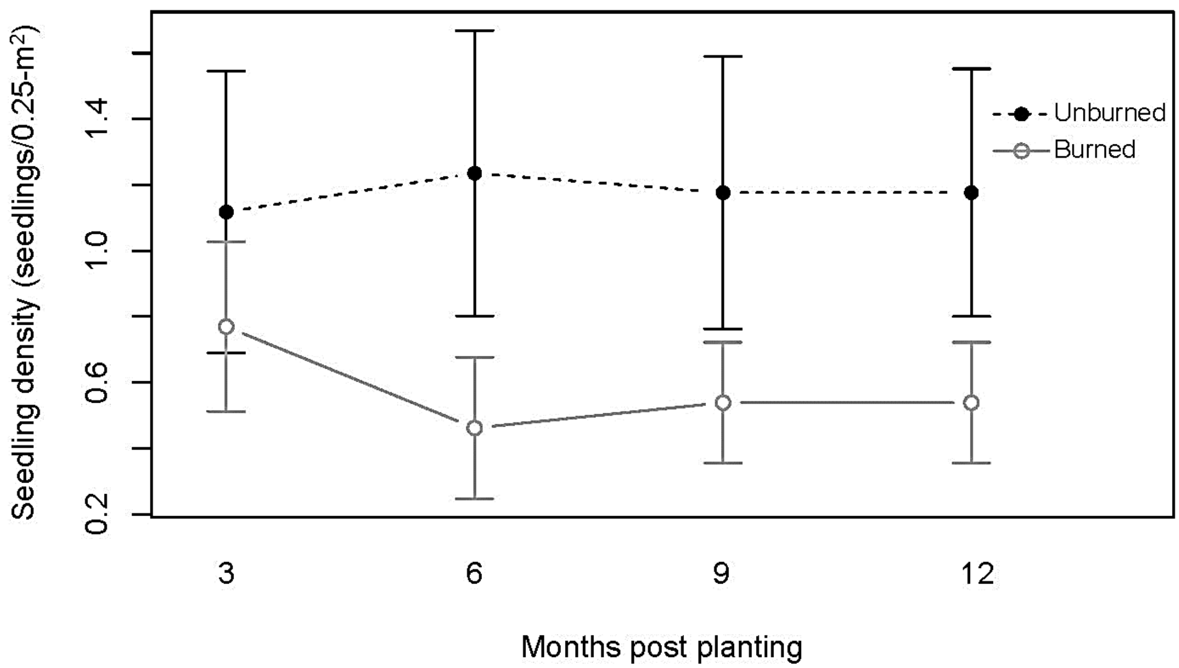

Figure 1-5. Tallow (*Triadica sebifera*) seedling establishment over time in unburned and burned plots in southern Mississippi, USA.

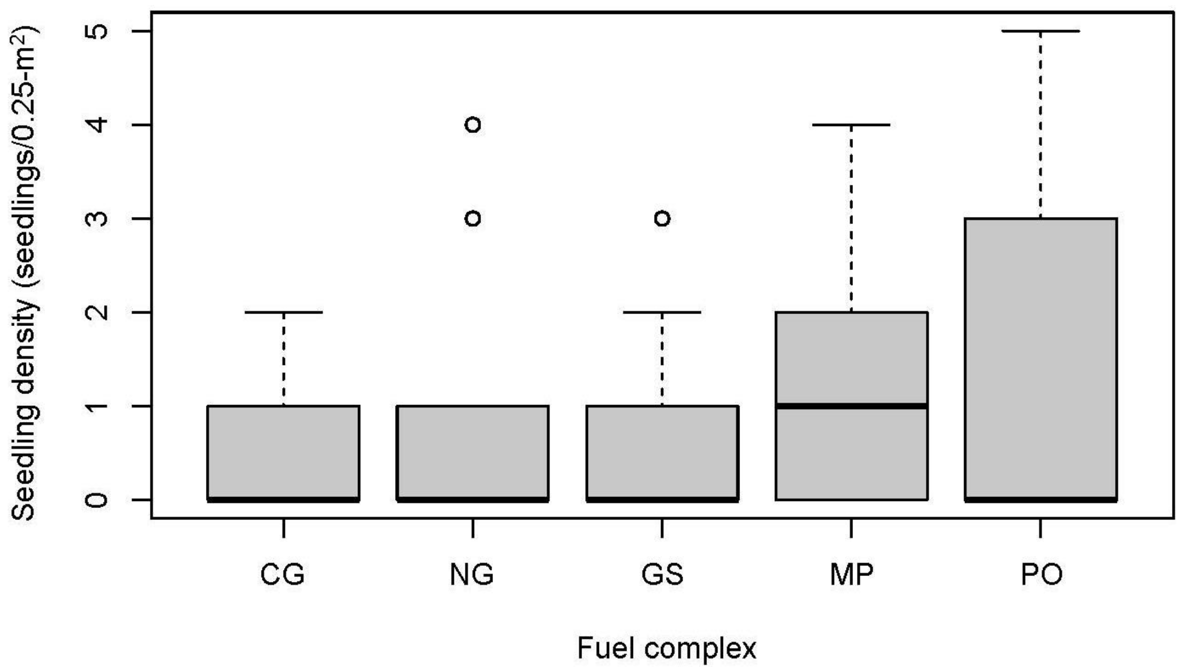

Figure 1-6. Tallow (*Triadica sebifera*) seedling establishment in cogongrass (CG), native grass (NG), mixed grass/shrub (GS), mixed pine (MP), and pine only (PO) fuel complexes found throughout our study sites in southern Mississippi, USA.

Table 1-2. Mean (sd) fuel loading (g/m^2) and percent contribution of pine, hardwood, grass, and shrub contribution of five fuel complexes common in Mississippi Sandhill Crane National Wildlife Refuge and Grand Bay National Estuarine Research Reserve, southern Mississippi, USA.

Fuel complex	Native grass	Mixed grass/shrub	Mixed pine	Pine only	Cogongrass
Seedling density (seedlings/0.25-m^2)	0.8 (1.3)	1.3 (1.4)	1.3 (1.4)	1.4 (1.9)	0.5 (0.72)
Fuel loading (g)	562.4 (233.1)	378.6 (189.3)	578.1 (87.6)	795.4 (204.2)	390.5 (185.1)
% pine	47.8 (26.2)	0	53.6 (36.4)	90.3 (5.3)	57.7 (26.2)
% hardwood	1.4 (0.5)	0	12.7 (12.9)	8.9 (5.0)	0.2 (0.6)

% grass/herb	33.4 (40.5)	53.9 (35.8)	33.0 (36.0)	0.5 (1.0)	37.6 (27.6)
% woody	19.7 (31.0)	46.1 (35.8)	0.7 (1.0)	0.3 (0.5)	4.4 (5.5)

Discussion

Seed viability

Our findings suggest that tallow seeds have limited (< 6-mo) viability in the soil seedbank in coastal flatwoods and savannas and scarification decreases viability only during this short post-dispersal window. Harper (1995) measured tallow seed viability of 10-15% after one year in the soil, 2-3 times higher than our estimates of ~5% over the same period. Seed tree provenance was shown to impact tallow germination and seedling survival (Cameron et al., 2000), so it is possible these same characteristics could drive changes in viability. Renne (2001) explored scarification effects on tallow seedling emergence and found acid-scarified seeds had the highest emergence and viability while unscarifed seeds had the lowest viability. This contrasts with our finding of unscarifed seeds remaining viable for longer; however, this was conducted in a greenhouse as opposed to the field. Temperature fluctuations, soil moisture, soil salinity, and disease could be responsible for our lower seed viability results (Zhang et al., 2019). Similarly, due to these circumstances, unscarified seeds with the waxy seed coat still in place may be more resistant to damage from these conditions, resulting in the higher initial viability we observed in our study.

Our study did not detect any differences in viability based on depth below the soil surface for either scarified or unscarified seeds at any time for the duration of this experiment. While our rapid decline in tallow seed viability may not be mirrored by Renne (2001), our project was unique in its approach to assessing seed viability *in situ* in southern Mississippi along the Gulf of Mexico coast compared to other studies that took place in a greenhouse or laboratory. While some studies (Conn et al., 2006; Mennan & Zandstra, 2006) found significant decreases in viability with increasing depth below the soil surface, our results did not demonstrate any differences in viability by depth.

One limitation of our study was the time it took from collecting seeds to outplanting them in Mississippi. We understand that in natural conditions, tallow fruit matures in early fall and begins dehiscence and seed dispersal soon after. While we collected mature seeds from tallow trees in October 2021, we were not able to plant them at our study site until May 2022. Despite this, we assessed the viability of a sample of tallow seeds immediately prior to outplanting and found that both scarified and unscarified seeds had similarly high viability (92 and 94%, respectively). It is possible that warmer conditions in summer could contribute to the observed rapid decline in seed viability compared to what might happen under natural seed dispersal circumstances; however, we do not believe differences in soil moisture contributed to the observed decline in viability as this remained similar across all collection periods.

Seedling establishment

Our results demonstrate that tallow early seedling establishment was low, around 1.18 and 0.58% for unburned and burned plots, respectively. We did not find any significant changes in the seedling density over time. Additionally, seedling establishment was similar among all five fuel types in plots that were burned during our experiment. Our findings of higher tallow seedling establishment in unburned areas mirror those of Burns & Miller (2004) where they observed higher tallow seedling establishment in unburned areas than in burned; however, they observed higher germination rates of 7.4 and 1% for unburned and burned, respectively. This difference could be attributed to the high salinity at our study site locations and tallow's apparent low tolerance to these conditions (Barrilleaux & Grace, 2000; Paudel & Battaglia, 2015). On the contrary, some studies observed increased tallow seedling establishment following a fire. Donahue (2006) found higher establishment in cleared areas that had been recently burned due to increased light and temperature oscillation that facilitate seedling establishment (Donahue et al., 2004; Nijjer, 2002). Fan et al. (2021b) observed higher seedling establishment in burned areas; however, most of these seedlings were not persistent and ultimately died after one year. Renne (2001) found no difference in tallow seedling establishment in five different coastal habitat types. While our fuel complexes represent variation in plant communities rather than distinct forest types, it mirrors these findings in that there was no observed difference in seedling establishment in any of our five identified fuel complexes.

As with the seed viability component of this study, we collected tallow seeds when they matured in October 2021 and did not outplant them in Mississippi until April 2022. This opens the potential for conditions outside of those ordinarily experienced by tallow seeds following dispersal that could impact the observed seedling establishment results. Additionally, we intended to test the effect of fuel loading on seedling establishment at both study sites. However, due to the unpredictable nature of burning National Wildlife Refuge land, half of our plots remained unburned. Fortunately, could still assess the impact of different fuel complexes on the burned plots, and we could also directly compare these to the unburned plots at our other study site.

Conclusion

Scarification does impact tallow seed viability and suggests a “lag phase” where unscarified seeds maintain higher viability than scarified seeds. However, regardless of scarification treatment, seed viability decreases rapidly for tallow seeds at all depths below the soil surface indicating that tallow's ability to establish itself in the soil seedbank may be limited. Additionally, tallow early seedling establishment was low across our study sites (~1%), but is further reduced by prescribed fire. These findings indicate that through appropriate management techniques such as frequent prescribed fire and the removal of mature, seed producing trees in the overstory, the threat of invasion from tallow can be mitigated.

References

Barrilleaux, T. C., & Grace, J. B. (2000). Growth and Invasive Potential of Sapium sebiferum (Euphorbiaceae) within the Coastal Prairie Region: The Effects of Soil and Moisture Regime. *American Journal of Botany*, *87*(8), 1099–1106. https://doi.org/10.2307/2656646

Burns, J., & Miller, T. (2004). Invasion of Chinese Tallow (Sapium sebiferum) in the Lake Jackson area, Northern Florida. *The American Midland Naturalist*, *152*, 410–417. https://doi.org/10.1674/0003-0031(2004)152[0410:IOCTSS]2.0.CO;2

Bruce, Katherine A.; Cameron, Guy N.; Harcombe, Paul A.; Jubinsky, Greg. 1997. Introduction, impact on native habitats, and management of a woody invader, the Chinese tallow tree, Sapium sebiferum (L.) Roxb. *Natural Areas Journal*. 17(3): 255-260.

Bruce, Katherine A. 1993. Factors affecting the biological invasion of the exotic Chinese tallow tree, Sapium sebiferum, in the Gulf Coast prairie of Texas. Houston, TX: University of Houston. 155 p. Thesis.

Cameron, G. N., Glumac, E. G., & Eshelman, B. D. (2000). Germination and Dormancy in Seeds of Sapium sebiferum (Chinese Tallow Tree). *Journal of Coastal Research*, *16*(2), 391–395.

Cameron, G. N., & Spencer, S. R. (1989). Rapid leaf decay and nutrient release in a chinese tallow forest. *Oecologia*, *80*(2), 222–228. https://doi.org/10.1007/BF00380155

Conn, J. S., Beattie, K. L., & Blanchard, A. (2006). Seed viability and dormancy of 17 weed species after 19.7 years of burial in Alaska. *Weed Science*, *54*(3), 464–470. https://doi.org/10.1614/WS-05-161R.1

Conway, W., Smith, L. M., & Bergan, J. F. (2000). Evaluating germination protocols for Chinese tallow (Sapium sebiferum) seeds. *Texas Journal of Science*, *52*, 267–270.

Conner, W. H., & Askew, G. R. (1993). Impact of Saltwater Flooding on Red Maple, Redbay, and Chinese Tallow Seedlings. *Castanea*, *58*(3), 214–219.

Conner, W. H. (1994). The Effect of Salinity and Waterlogging on Growth and Survival of Baldcypress and Chinese Tallow Seedlings. *Journal of Coastal Research*, *10*(4), 1045–1049.

Daubenmire, R. 1959. A Canopy-Coverage Method of Vegetational Analysis. Northwest Science 33(1): 43-64.

Donahue, C., Rogers, W. E., & Siemann, E. (2004). Effects of temperature and mulch depth on Chinese tallow tree (Sapium sebiferum) seed germination. *Texas Journal of Science*, *56*, 347–356.

Donahue. (2006). *Restoring an Invaded Prairie by Mulching Live Sapium sebiferum (Chinese Tallow Trees): Effects of Mulch on Sapium Seed Germination*. https://bioone.org/journals/natural-areas-journal/volume-26/issue-3/0885-8608(2006)26%5B244%3ARAIPBM%5D2.0.CO%3B2/Restoring-an-Invaded-Prairie-by-Mulching-Live-Sapium-sebiferum-Chinese/10.3375/0885-8608(2006)26[244:RAIPBM]2.0.CO;2.full

Erickson, H. E., & White, R. (2007). *Invasive plant species and the Joint Fire Science Program.* (PNW-GTR-707; p. PNW-GTR-707). U.S. Department of Agriculture, Forest Service, Pacific Northwest Research Station. https://doi.org/10.2737/PNW-GTR-707

Fan, Z., Song, A., Dong, L., Alexander, H. D., Yang, S., Cheng, N., & Pitchford, J. L. (2021a). Fire effects on post-invasion spread of Chinese tallow (Triadica sebifera) in wet pine flatwood ecosystems in the southeastern United States. *Forest Ecology and Management*, *500*, 119658. https://doi.org/10.1016/j.foreco.2021.119658

Fan, Z., Yang, S., Cheng, N., Liu, X., Song, A., & Dong, L. (2021b). Invasibility of fire-managed ecosystems to the Chinese tallow tree (Triadica sebifera) in the lower Gulf Coastal Plain, USA: Mechanisms and key factors at the landscape level. *Forest Ecology and Management*, *479*, 118539.

Fan, Z., Yang, S., Loewenstein, N. J., Cheng, N., Nepal, S., Pitchford, J. L., Chappell, J., & Stone, D. (2021c). Modeling spatial variations of the invasibility of slash pine flatwoods to Chinese tallow (Triadica sebifera) invasion: Mechanisms and key factors at the microscale. *Forest Ecology and Management*, *482*, 118798. https://doi.org/10.1016/j.foreco.2020.118798

Fan, Z., Yang, S., & Liu, X. (2018). Spatiotemporal Patterns and Mechanisms of Chinese Tallowtree (Triadica sebifera) Spread along Edge Habitat in a Coastal Landscape, Mississippi, USA. *Invasive Plant Science and Management*, *11*(3), 117–126. https://doi.org/10.1017/inp.2018.21

Fan, Z. (2018). Spatial Analyses of Invasion Patterns of Chinese Tallow (Triadica sebifera) in a Wet Slash Pine (Pinus elliottii) Flatwood in the Coastal Plain of Mississippi, USA. *Forest Science*, *64*(5), 555–563. https://doi.org/10.1093/forsci/fxy014

Flory, S. L., Dillon, W., & Hiatt, D. (n.d.). Interacting global change drivers suppress a foundation tree species. *Ecology Letters*, *n/a*(n/a). https://doi.org/10.1111/ele.13974

Fish and Wildlife Service (2010). “Mississippi Sandhill Crane National Wildlife Refuge: Unique Habitats.” Retrieved October 12, 2021, from https://www.fws.gov/mississippisandhillcrane/habitats.html

FNAI (Florida Natural Areas Inventory). 2010. Guide to the natural communities of Florida: 2010 edition. Florida Natural Areas Inventory, Tallahassee, FL, US. https://doi.org/10.1016/j.foreco.2020.118539

Frost, C.C. 1998. Presettlement fire frequency regimes of the United States: a first approximation. Pages 70-81 in T.L. Pruden and L.A. Brennan, editors. Fire in Ecosystem Management: Shifting the Paradigm from Suppression to Prescription. Tall Timbers Fire Ecology Conference Proceedings, No. 20. Tall Timbers Research Station, Tallahassee, Florida.

Gabler, C. A., & Siemann, E. (2013). Rapid ontogenetic niche expansions in invasive Chinese tallow tree permit establishment in unfavourable but variable environments and can be exploited to streamline restoration. *Journal of Applied Ecology*, *50*(3), 748–756. https://doi.org/10.1111/1365-2664.12071

Grace, J. (1998). Can prescribed fire save the endangered coastal prairie ecosystem from Chinese tallow invasion. *Endangered Species Update*, *15*.

Grace, James B.; Smith, Melinda D.; Grace, Susan L.; Collins, Scott L.; Stohlgren, Thomas J. 2001. Interactions between fire and invasive plants in temperate grasslands of North America. In: Galley, Krista E. M.; Wilson, Tyrone P., eds. Proceedings of the invasive species workshop: The role of fire in the control and spread of invasive species; Fire conference 2000: the first national congress on fire ecology, prevention, and management; 2000 November 27 - December 1; San Diego, CA. Misc. Publ. No. 11. Tallahassee, FL: Tall Timbers Research Station: 40-65.

Grace, James B.; Allain, Larry K.; Baldwin, Heather Q.; Billock, Arlene G.; Eddleman, William R.; Given, Aaron M.; Jeske, Clint W.; Moss, Rebecca. 2005. Effects of prescribed fire in the coastal prairies of Texas. USGS Open-File Report 2005-1287. Reston, VA: U.S. Department of the Interior, Fish and Wildlife Service, Region 2; U.S. Geological Survey. 46 p.

Gan, Jianbang; Miller, James H.; Wang, Hsiaohsuan; Taylor, John W., Jr. 2009. Invasion of tallow tree into southern US forests: influencing factors and implications for mitigation. Canadian Journal of Forest Research. 39: 1346-1356.

Groeschl, D. A., Johnson, J. E., & Smith, D. W. (1993). Wildfire Effects on Forest Floor and Surface Soil in a Table Mountain Pine-Pitch Pine Forest. *International Journal of Wildland Fire*, *3*(3), 149–154. https://doi.org/10.1071/wf9930149

Harper, M.G., 1995. A model of the invasion of Chinese tallow tree (Sapium sebiferum (L.) Roxb.) into a bottomland-hardwood forest in Louisiana. M.S. thesis, Tulane University, New Orleans, Louisiana.

Hibdon, Lisa M. 2005. Litter dynamics and detrital food webs of native and Chinese tallow stands in a Louisiana bottomland hardwood forest. New Orleans, LA: University of New Orleans. 85 p.

Hoffmann, W. A., Adasme, R., Haridasan, M., T. de Carvalho, M., Geiger, E. L., Pereira, M. A. B., Gotsch, S. G., & Franco, A. C. (2009). Tree topkill, not mortality, governs the dynamics of savanna–forest boundaries under frequent fire in central Brazil. *Ecology*, *90*(5), 1326–1337. https://doi.org/10.1890/08-0741.1

Jubinsky, Greg. 1995. Chinese tallow (Sapium sebiferum). Report No. TSS-93-03. Tallahassee, FL: Florida Department of Environmental Protection, Bureau of Aquatic Plant Management. 12 p. [54504]

Lin, W. C.; Chen, A. C.; Tseng, C. J.; Huang, S. G. 1958. An investigation and study of Chinese tallow tree in Taiwan (Sapium sebiferum Roxb). Bulletin of Taiwan Forestry Research Institute. 57: 32-37.

Mennan, H., & Zandstra, B. H. (2006). The Effects of Depth and Duration of Seed Burial on Viability, Dormancy, Germination, and Emergence of Ivyleaf Speedwell (Veronica hederifolia). *Weed Technology*, *20*(2), 438–444. https://doi.org/10.1614/WT-05-090R.1

Midgley, J. J., Kruger, L. M., & Skelton, R. (2011). How do fires kill plants? The hydraulic death hypothesis and Cape Proteaceae “fire-resisters.” *South African Journal of Botany*, *77*(2), 381–386. https://doi.org/10.1016/j.sajb.2010.10.001

Miller, James H. 2003. Nonnative invasive plants of southern forests: A field guide for identification and control. Gen. Tech. Rep. SRS-62. Asheville, NC: U.S. Department of Agriculture, Forest Service, Southern Research Station. 93 p.

Mohamed-Yasseen, Y., Barringer, S. A., Splittstoesser, W. E., & Costanza, S. (1994). The Role of Seed Coats in Seed Viability. *Botanical Review*, *60*(4), 426–439.

Moore, R.P. (1985) Handbook on Tetrazolium Testing. International Seed Testing Association, Zurich.

Nijjer, S., Lankau, R., Rogers, W. E., & Siemann, E. (2002). Effects of temperature and light on Chinese tallow (Sapium sebiferum) and Texas sugarberry (Celtis laevigata) seed germination. *Texas Journal of Science*, *54*, 63–68.

NOAA *NCEI U.S. Climate Normals Quick Access*. (n.d.). Retrieved October 4, 2021, from https://www.ncei.noaa.gov/access/us-climate-normals/#dataset=normals-annualseasonal&timeframe=30&location=MS&station=US1MSJC0012

NRCS *Web Soil Survey—Home*. (n.d.). Retrieved October 5, 2021, from https://websoilsurvey.sc.egov.usda.gov/App/HomePage.htm

Paudel, S., & Battaglia, L. L. (2015). The role of light, soil and human factors on the probability of occurrence of an invasive and three native plant species in coastal transitions of coastal Mississippi, USA. *Journal of Plant Ecology*, *8*(5), 491–500. https://doi.org/10.1093/jpe/rtu045

Pile, L. S., Wang, G. G., Stovall, J. P., Siemann, E., Wheeler, G. S., & Gabler, C. A. (2017). Mechanisms of Chinese tallow (Triadica sebifera) invasion and their management implications – A review. *Forest Ecology and Management*, *404*, 1–13. https://doi.org/10.1016/j.foreco.2017.08.023

Pimentel, D., Zuniga, R., & Morrison, D. (2005). Update on the environmental and economic costs associated with alien-invasive species in the United States. *Ecological Economics*, *52*(3), 273–288. https://doi.org/10.1016/j.ecolecon.2004.10.002

R Core Team (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Renne, I. J., Spira, T. P., & Bridges, W. C. (2001). Effects of Habitat, Burial, Age and Passage through Birds on Germination and Establishment of Chinese Tallow Tree in Coastal South Carolina. *The Journal of the Torrey Botanical Society*, *128*(2), 109–119. https://doi.org/10.2307/3088733

Renne, I. J., Barrow, W. C., Randall, L. A. J., & Bridges, W. C. (2002). Generalized avian dispersal syndrome contributes to Chinese tallow tree (Sapium sebiferum, Euphorbiaceae) invasiveness. *Diversity and Distributions*, *8*(5), 285–295. https://doi.org/10.1046/j.1472-4642.2002.00150.x

Rockwood, D. L.; Geary, T. F. 1991. Growth of 19 exotic and two native tree species on organic soils in southern Florida. In: Center, Ted D.; Doren, Robert F.; Hofstetter, Ronald L.; Myers, Ronald L.; Whiteaker, Louis D., eds. Proceedings of the symposium on exotic pest plants; 1988 November 2-4; Miami, FL. Tech. Rep. NPS/NREVER/NRTR-91/06. Washington, DC: U.S. Department of the Interior, National Park Service: 283-302.

Scheld, Herbert W.; Cowles, Joe R. 1981. Woody biomass potential of the Chinese tallow tree. *Economic Botany*. 35(4): 391-397.

Siemann, E., & Rogers, W. (2006). Recruitment Limitation, Seedling Performance and Persistence of Exotic Tree Monocultures. *Biological Invasions*, *8*, 979–991. https://doi.org/10.1007/s10530-005-0825-9

Susko, D., Mueller, J., & Spears, J. (2011). An evaluation of methods for breaking seed dormancy in kudzu (Pueraria lobata). *Canadian Journal of Botany*, *79*, 197–203. https://doi.org/10.1139/b00-153

Wang, H.-H., Grant, W. E., Swannack, T. M., Gan, J., Rogers, W. E., Koralewski, T. E., Miller, J. H., & Taylor, J. W. (2011). Predicted range expansion of Chinese tallow tree (*Triadica sebifera*) in forestlands of the southern United States: Range expansion of Chinese tallow tree. *Diversity and Distributions*, *17*(3), 552–565. https://doi.org/10.1111/j.1472-4642.2011.00760.x

Yang, S., Fan, Z., Liu, X., Ezell, A. W., Spetich, M. A., Saucier, S. K., Gray, S., & Hereford, S. G. (2019). Effects of Prescribed Fire, Site Factors, and Seed Sources on the Spread of Invasive Triadica sebifera in a Fire-Managed Coastal Landscape in Southeastern Mississippi, USA. *Forests*, *10*(2), Article 2. https://doi.org/10.3390/f10020175

Zhang, Z., Gao, P., Dai, W., Song, X., Hu, F., & Qiang, S. (2019). Effect of tillage and burial depth and density of seed on viability and seedling emergence of weedy rice. *Journal of Integrative Agriculture*, *18*(8), 1914–1923. https://doi.org/10.1016/S2095-3119(19)62583-9

Chapter 2: Impact of increasing Chinese tallow (*Triadica sebifera*) litter contribution to the fuelbed on forest flammability and seed viability

Abstract

Chinese tallow (*Triadica sebifera*) is an invasive exotic tree species that has become established in endangered longleaf and slash pine (*Pinus palustris*, *P. elliottii*) savannas and flatwoods along the Gulf of Mexico coast in the southeast United States. These pine forests require frequent fire to maintain their structure, health, and integrity, and as a result, prescribed burning is a common practice in these ecosystems. Unfortunately, tallow potentially threatens this process by creating less flammable conditions that favor its proliferation to the detriment of fire-dependent ecosystems. For this study, we assessed how increasing proportions of tallow litter, from heavily invaded to uninvaded, influence forest flammability metrics such as rate of spread (ROS), fire temperature, flame height, and area burned. To achieve this, we collected tallow and pine litter and created four replicate plots (4-m^2) of 5 differing proportions of tallow: 1) 100% tallow, 0% pine, 2) 75% tallow, 25% pine, 3) 50% tallow, 50% pine, 4) 25% tallow, 75% pine, and 5) 0% tallow and 100% pine. In addition to flammability, we tested if tallow seed viability is impacted by changes in pre-burn fuelbed (FB), fire (F), or a combination of fuelbed and fire (FBF) differences due to increasing tallow contribution to the fuelbed. We determined seed viability using the tetrazolium (TZ) test by allowing seed embryos to imbibe in a 1% TZ solution for 72 hours, and seeds stained red were presumed to be viable. Plots were established in December 2022 with litter and FB and FBF seed bags to allow litter and seeds to experience field conditions prior to burning. Before experimentally burning plots in March 2023, we replaced the FB seed bags with F seed bags to isolate fire-only impacts. Our results show a negative linear relationship between proportion tallow contribution to the fuelbed and ROS, temperature, flame

height, and area burned. Seed viability was not different regardless of seed bag treatment (FB, F, FBF) or proportion tallow contribution to the fuelbed suggesting that tallow seeds are resistant to the effects of fire under the conditions we were able to create. These results demonstrate the impact tallow litter has on native fire-dependent ecosystem flammability. Tallow litter's fire suppressing abilities may foster its persistence and spread across the landscape, especially in areas not frequently managed with fire where tallow litter can accumulate in the fuelbed.

Introduction

Chinese tallow tree (*Triadica sebifera*; hereafter: tallow) is an invasive exotic tree species introduced in the 18th century (Pile et al., 2017). Today, tallow threatens the health and integrity of many ecosystems across the southeast U.S., including fire-dependent longleaf and slash pine (*Pinus palustris, P. elliottii*) flatwoods and savannas that require frequent, low intensity, surface fires every 1-3 years to maintain their structure. While tallow's ability to spread rapidly and transform ecosystems by forming a dense midstory is known, the effect that its litter contribution to the fuelbed on fire behavior has not been explored as it relates to changes in forest flammability and tallow proliferation.

Different fuel types and canopy structures can alter fire temperature, and tallow litter is suspected to reduce the rate of spread and temperature of fire (Groeschl et al., 1993, Grace, 1998). Since fire behavior and forest flammability are primarily impacted by leaf litter that comprises the fuelbed in closed canopy ecosystems (Varner et al., 2015; Kreye et al., 2023), additional litter inputs from tallow could alter the native fire regime. Tallow litter decomposes rapidly and forms dense, tightly packed mats on the forest floor that maintain high moisture (Cameron & LaPoint, 1978; Cameron & Spencer, 1989; Zhang et al., 2017), and leaf litter with high moisture content reduces the probability of a successful fire (Rothermel, 1972). Additionally, rapid litter decomposition rates can further reduce fuel loads and add to fuel moisture content (Babl-Plauche et al., 2022). Thin, flat leaves tend to hold more moisture and are less likely to carry fire (Schwilk and Caprio, 2011; Cornwell et al., 2015; Babl et al., 2020). Additionally, in just a few years, tallow can create a dense forest midstory with high a leaf area that alters the ecosystem's microclimatic conditions, which has the potential of reducing forest flammability (Grace, 1998; Siemann & Rogers, 2006). Specific leaf litter traits such as leaf curl, thickness, and moisture content alter the degree of a given species' flammability (McDaniel et al., 2021). Changes in fuel flammability characteristics ultimately reduce the spread of fire in pyric forests. The process of transitioning from a state of high flammability to a cooler, moister, and less flammable state is called mesophication (Nowacki & Abrams, 2008; Alexander et al., 2021). Thus, as tallow encroaches into an ecosystem, there will likely be changes in fire behavior resulting from the accumulation of potentially less flammable litter and an altered microclimate resulting in reduced ecosystem quality.

Tallow spreads primarily through seeds, and seeds that have the waxy coat removed via scarification tend to germinate more readily (Renne et al., 2001). Because fire can scarify the seeds of other invasive species (Susko et al., 2011), we suspect that fire may do the same for tallow seeds, yet there is currently no information on the effect of prescribed fire on tallow seed viability. One mature tallow tree can produce up to 100,000 seeds in a single growing season, making it difficult to control its spread (Lin et al., 1958; Conway et al., 2000). Changes in fire temperature resulting from different fuels may influence whether tallow seeds survive the burn or not. Since leaf litter comprising the fuelbed is an important factor impacting fire behavior in closed canopy forests, areas with increased litter inputs from tallow may promote less effective fires that facilitate positive feedback, leading to more favorable conditions for tallow seeds.

For this study, we sought to investigate how increasing tallow litter contribution to the fuel bed influences fire behavior characteristics such as rate of spread, flame height, maximum temperature, and consumption (area burned). Additionally, we wanted to understand what impact these changes would have on tallow seed viability. Specifically, we hypothesized that as the tallow litter contribution to the fuel bed increases, the rate of spread, flame height, maximum temperature, and consumption would decrease compared to plots with higher proportions of pine.

We also hypothesized that plots with high proportions of tallow litter will have lower fire temperature, and, as a result, will have the highest seed viability post burn. If tallow litter can reduce flammability, it is possible that a feedback loop can be created that facilitates its own spread by protecting its seeds and reducing damage from fire, resulting in further invasion in of tallow within fire-dependent ecosystems and reduced effectiveness of fire as a key natural process.

Methods

Study sites

We collected tallow seeds and leaf litter from Mississippi Sandhill Crane National Wildlife Refuge (NWR) in Jackson County, MS (30.475°N, 88.783°W). Historically, this study site contained many different forest types, but is dominated by longleaf and slash pine (*Pinus palustris* & *P. elliottii*) flatwoods and savannahs with a low overstory tree density and canopy cover. Fuels in these flatwoods are primarily from inputs of pine litter with sparse grass and shrubs, but fuel loads are generally higher in savannas because of higher light levels and lower pine leaf litter inputs. Unfortunately, Mississippi Sandhill Crane NWR also has extensive biological invasion from tallow (Fan, 2018; Fan et al., 2021a; Fan et al., 2021b). As a result, this site was selected due to the high degree of invasion by tallow, often in dense clusters, that aided in both the collection of seeds and leaf litter for this experiment.

For this study, a series of experimental burns took place at Auburn University's Mary Olive Thomas Demonstration Forest (MOT), Auburn, Alabama, U.S.A. We chose to establish our leaf litter plots here due to the proximity to Auburn University and similar canopy structure of the MOT forest to the study site in Mississippi. Both forest types have a sparse or absent midstory. Rainfall was lower at MOT compared to Mississippi Sandhill Crane NWR at ~134 cm compared to ~175 cm annually with peak rainfall in the summer months for both sites (NOAA, 2021). Annual high and low temperatures were 26 °C in July and 12 °C in January at Mississippi Sandhill Crane NWR and 32 °C high in July and 4 °C at MOT.

Litter flammability characteristics

Leaf litter collection:

We collected tallow leaf litter in December 2022 from Mississippi Sandhill Crane NWR in southern Mississippi, US using a leaf blower to first gather and pile the leaves and then a rake to put them in large paper bags. After transporting the leaf litter to Auburn, we stored it in large paper bags in a climate-controlled room at 18° C for two weeks until the litter achieved a constant air-dry weight. Pine (*Pinus* spp.) needle bales were also purchased in December 2022 from a local hardware store. Pine litter was stored under the same conditions as tallow to achieve a constant air-dry weight. Then, in January 2023, we established twenty 4-m^2 plots in four replicate blocks of five fuel treatments containing different proportions of pine and tallow litter at MOT. Each block contained the following proportions of pine to tallow litter by weight to simulate increasingly invaded ecosystems. These complexes, from heavily uninvaded to invaded, included: 1) 100% pine, 2) 25% tallow and 75% pine, 3) 50% tallow and 50% pine, 4) 75% tallow and 25% pine and 5) 100% tallow (Figure 2-1 A-E). We measured mean fuel loading from fuelbed samples collected at our study sites from pine dominated flatwoods (“pine only”) where leaf litter dominates the fuelbed in southern Mississippi in March 2021. Fuel loading was kept constant across all plots with the same 0.75 kg/m^2 to replicate conditions found at our Mississippi Study sites, but in differing proportions of pine to tallow.

Experimental burn:

In February 2023, 50 days after the plots were established, we conducted experimental dormant season burns, as this is the time of year many prescribed fires are implemented in the region. Immediately prior to burning the plots, we placed four pyrometers containing six Tempilaq (Tempil, S Plainfield, NJ, U.S.A.) temperature sensitive paints ranging from 79° C to 510° C in each plot next to the seed bags to estimate the maximum temperature achieved by the fire at each burned seed bag (Nation et al., 2021; Cabrera et al., 2023). While pyrometers only estimate a maximum temperature at one point in the plot rather than the actual fire temperature, they can still be effective in measuring mean maximum temperature for a burn (Iverson et al., 2004). Pyrometers are useful for comparing differences between treatments, especially at small scale experimental burns (Nation et al., 2021; Cabrera et al., 2023). Additionally, we collected a small sample of litter from each plot to determine the percent moisture on the day of the burn.

Using a drip torch (3:2 diesel:gasoline), we ignited each plot using a headfire firing technique. Once the plot's litter was carrying the fire, we started a timer and noted the time when the fire reached the middle of the plot, end of the plot, and when it extinguished to assess rate of spread (ROS, m/min) and flaming time (minutes). If the fire went out prior to reaching the end of the plot, we noted the flame time and measured the total distance (cm) traveled to determine fire ROS. Additionally, average flame height was estimated every 30 seconds using a measuring pole with 10 cm index marks on the edge of each plot.

Figure 2-1. Leaf litter flammability experimental burn plots containing 0, 25, 50, 75, and 100 percent Chinese tallow (Triadica sebifera) litter contribution to the fuelbed (A, B, C, D, E; respectively) post-burn at Mary Olive Thomas Demonstration Forest, Auburn, AL, USA.

Seed viability

Tallow seeds were collected in October 2021 from Mississippi Sandhill Crane NWR from six trees aging from 12 – 26 years old and size from 13 – 30 cm DBH. To understand potential drivers of change in seed viability, we independently examined the effects of pre-fire fuelbed conditions, fire, and a combination of fuelbed and fire on the viability of tallow seeds in different fuel combinations.

Inside each plot, we placed four wire mesh bags containing 20 tallow seeds each at the center of each four quadrants in January 2023. Two bags were used to assess the impact of changes to the pre-fire fuelbed (FB) caused by increasing proportions of tallow litter on seed viability. We collected the FB bags prior to burning in February 2023 and replaced them with two additional bags of tallow seeds stored off-site unimpacted by the pre-fire fuelbed to assess only the impact of fire (F) on tallow seed viability. The other two bags we initially placed in January remained in the plots for the burn in March to determine the combined impact of pre-fire fuelbed and fire (FBF) on seed viability.

Since our initial 4-m^2 plot burns did not reach high enough temperatures to reduce tallow seed viability, we conducted additional burns with fuels clipped from complexes common throughout our study sites in southern Mississippi that were collected for quantifying fuel components in *Chapter 1*. These include: 1) native grasses, 2) mixed native grasses and inkberry (*Ilex glabra*), 3) mixed pine (*Pinus* spp.) litter and native grasses, 4) pine litter, and 5) cogongrass (*Imperata cylindrica*). We created three replicate plots of each fuel type with one seedbag containing 20 tallow seeds in each plot. Additionally, we placed a modified pyrometer with a higher maximum temperature paint next to the seedbag which allowed us to detect fire temperatures up to 816 °C. We experimentally burned these plots in May 2023 to determine what impact different fire temperatures resulting from each complex had on seed viability. While these plots did not reflect the same structure of the particular fuel complex they were collected from, they still offered different fuel loading and contribution of grass, woody, and pine inputs that resulted in different maximum temperatures.

Immediately following the burns, we collected tallow seed bags and transported them back to Auburn University to assess post burn condition and seed viability using the tetrazolium chloride (TZ) test (Renne et al. 2001, Conn et al, 2006, Moore, 1985). First, tallow seeds in bags that were burned were checked for scorch marks and signs of charring. If the seed had at least 50% of the seedcoat charred, it was classified as having been charred by the fire. Tallow seeds were then allowed to imbibe in a 1% TZ solution for three days. Following soaking, the seeds were dissected, and any seeds stained red were assumed to be viable and seeds that did not change color were considered dead (Figure 2-2).

Figure 2-2. Viable (red, left) and dead (white, right) tallow (*Triadica sebifera*) seed embryo following soaking in a 1% tetrazolium chloride solution for 72 hours.

Leaf litter traits

To help explain potential differences in fire behavior on flammability of plots with differing amounts of tallow, we examined the individual leaf traits of this species. A random sample of thirty tallow leaves were collected from our tallow litter to assess leaf curling, exposed leaf area (cm^2), leaf one-sided surface area (cm^2), leaf perimeter (cm), leaf thickness (mm), and leaf oven dry mass (g).

To determine leaf curling, each leaf was set on the table without flattening. The distance from the table to the tallest point reached by the leaf was recorded. The exposed leaf area was determined by scanning the leaf in its current, unflattened, state. The leaf one-sided surface area was taken after relaxing the leaf in water, flattening in a press, and rescanning. This same scan was used to gather leaf perimeter data. The software ImageJ (National Institutes of Health, LOCI, University of Wisconsin-Madison, Madison, WI, USA) was used to estimate the area and perimeter values from scanned jpeg files of each leaf. We determined leaf thickness in two ways: midvein thickness at the middle of the leaf and leaf edge thickness. Following the second scan, we cut the leaf in half with a razor at the midpoint and measured the midvein and edge with calipers. Finally, the leaf samples were placed in an oven at 60 ° C for 48 hours to dry to a consistent dry mass and weighed following this drying period.

Data analysis:

Using linear regressions, we tested for differences in fire behavior (flame height, area burned, rate of spread, and temperature) between different amounts of tallow contribution to the fuelbed (0, 25, 50, 75, and 100% tallow contribution) with a significance of $\alpha = 0.05$. Since fire behavior metrics were highly correlated, a principal component analysis (PCA) was performed to understand changes in overall plot flammability. Following this, a linear regression was conducted to assess the impact of proportion tallow litter contribution to the fuelbed principal component 1 values. We tested for differences in seed viability and seed char (response variables) based on two factors: seed bag treatment (FB, FBF, and F) and tallow contribution to the fuelbed using a two-way analysis of variance (ANOVA). Any higher order interactions were retested using a linear mixed effects model using plot as a random effect. All statistical analyses were performed with R-4.2.1 (R Core Team 2021).

Results

Experimental Burns

In our experimental burns, as tallow litter contribution to the fuel bed increased, we observed a negative linear relationship for each of the metrics of fire behavior. Plots with increased proportions of tallow litter burned with a slower rate of spread, lower temperature, smaller area burned, and shorter flame height. Flame height ($R^2 = 0.80$, $p < 0.0001$, figure 2-3A) and area burned ($R^2 = 0.79$, $p < 0.0001$, figure 2-3B) were affected most by tallow litter and decreased as the proportion of tallow litter in the fuelbed increased. Rate of spread ($R^2 = 0.39$, $p < 0.0001$, figure 2-3C) and temperature ($R^2 = 0.32$, $p < 0.0001$, figure 2-3D) were not impacted by tallow litter as heavily, but they also decreased as proportion tallow litter contribution to the fuelbed increased.

Our PCA comprised of flame height, area burned, rate of spread, and temperature resulted in four principal components that were confirmed with the Bartlett test of sphericity (2,032.9, $p < 0.001$). Principal component 1 and principal component 2 explained 74.2% and 13.4% of the variation, respectively, and principal component 3 and principal component 4 explained 10.8% and 1.6% of variation, respectively (Table 2-1). Plots with more flammable, pine dominated litter had lower, more negative principal component 1 values compared to less flammable, tallow dominated plots with higher, more positive principal component 1 values (Figure 2-4). A linear model of principal component 1 and proportion tallow leaf litter resulted in a significant relationship between plot flammability and tallow litter contribution to the fuelbed ($R^2 = 0.76$, $P < 0.001$, Figure 2-5).

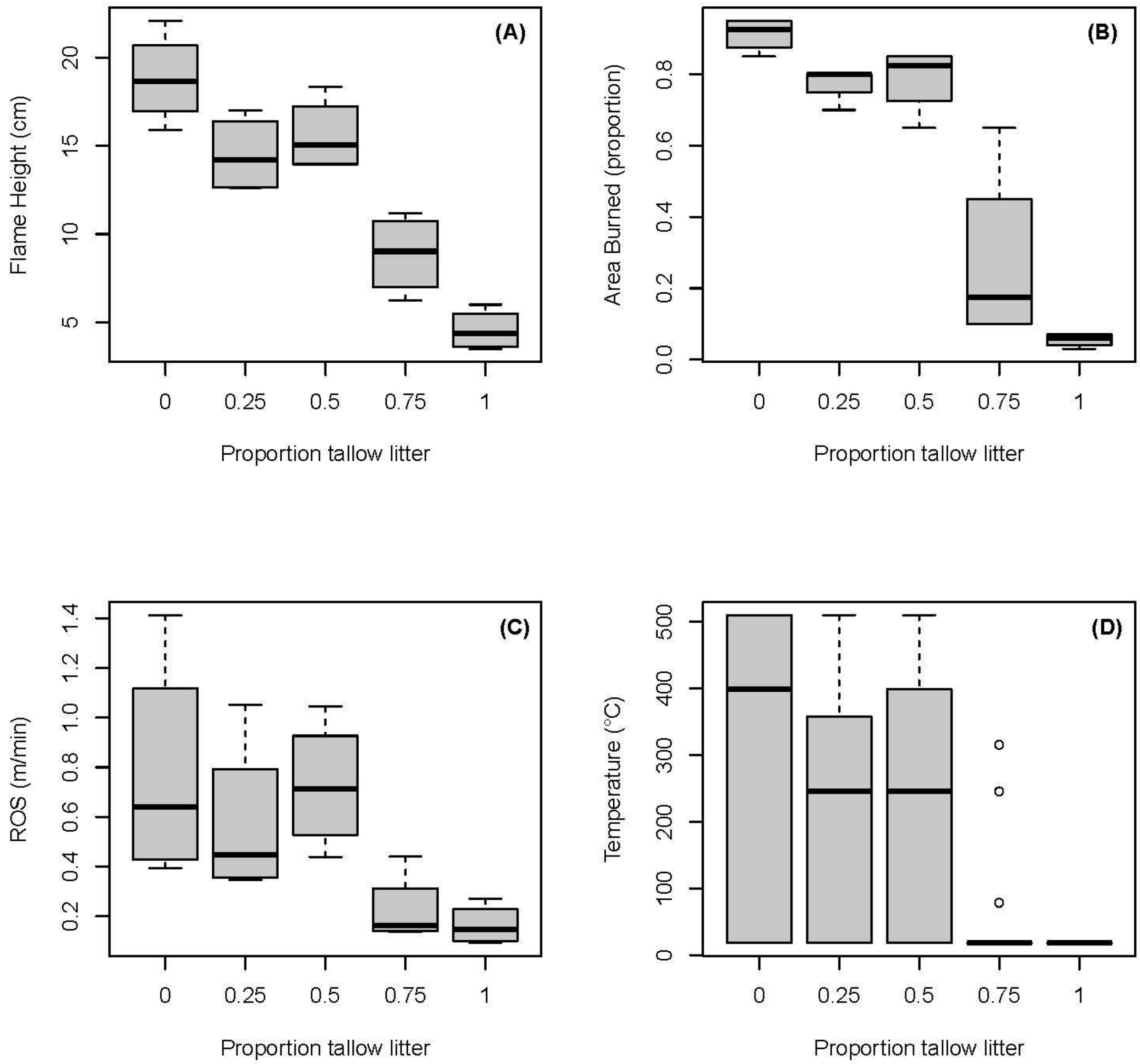

Figure 2-3. Relationship of proportion tallow and (A) flame height, (B) proportion area burned, (C) rate of spread (ROS), (D) and temperature measured during plot-level experimental burns of mixtures of increasing proportion of tallow (*Triadica sebifera*) litter to native pine (*Pinus* spp.) at Mary Olive Thomas Demonstration Forest, Auburn, AL, USA.

Table 2-1. Eigenvectors from principal component analysis (PCA) of fire behavior metrics from experimental burns of differing proportions of tallow (*Triadica sebifera*) litter at Mary Olive Thomas Demonstration Forest, Auburn, AL, USA.

Fire behavior metric	PC1	PC2	PC3	PC4
Area burned	-0.55	0.24	-0.22	-0.77
Temperature	-0.44	-0.89	0.14	-0.01
Flame height	-0.52	0.16	-0.59	0.59
Rate of Spread	-0.48	0.35	0.77	0.24
% Variance explained	74.22	13.41	10.80	1.57

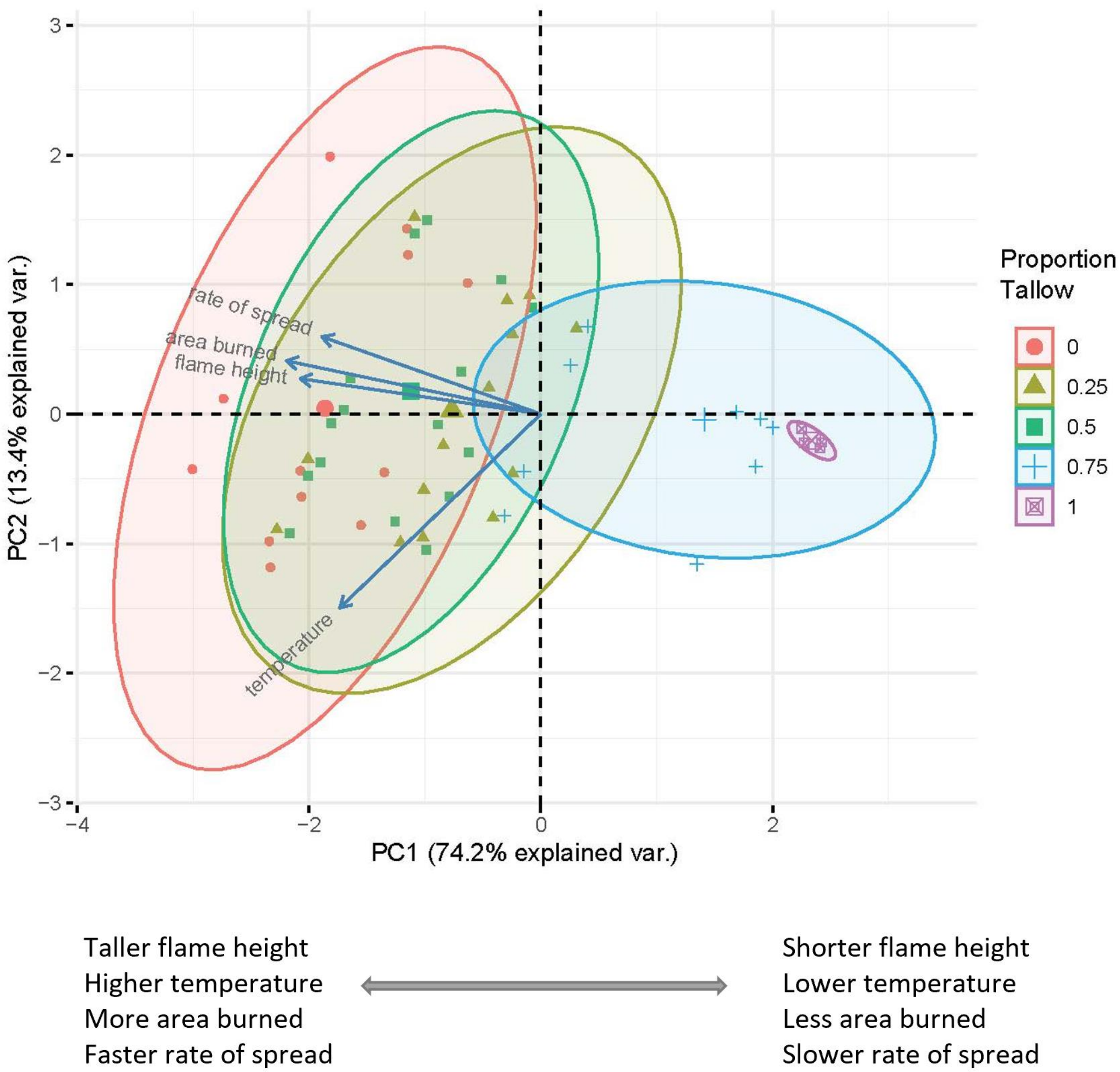

Figure 2-4. Principal component analysis (PCA) plot for fire behavior metrics measured during experimental burns of mixtures of increasing proportion of tallow (*Triadica sebifera*) litter relative to native pine (*Pinus* spp.) fuel with 95% confidence ellipses. Larger symbols represent multiple plots with the same value.

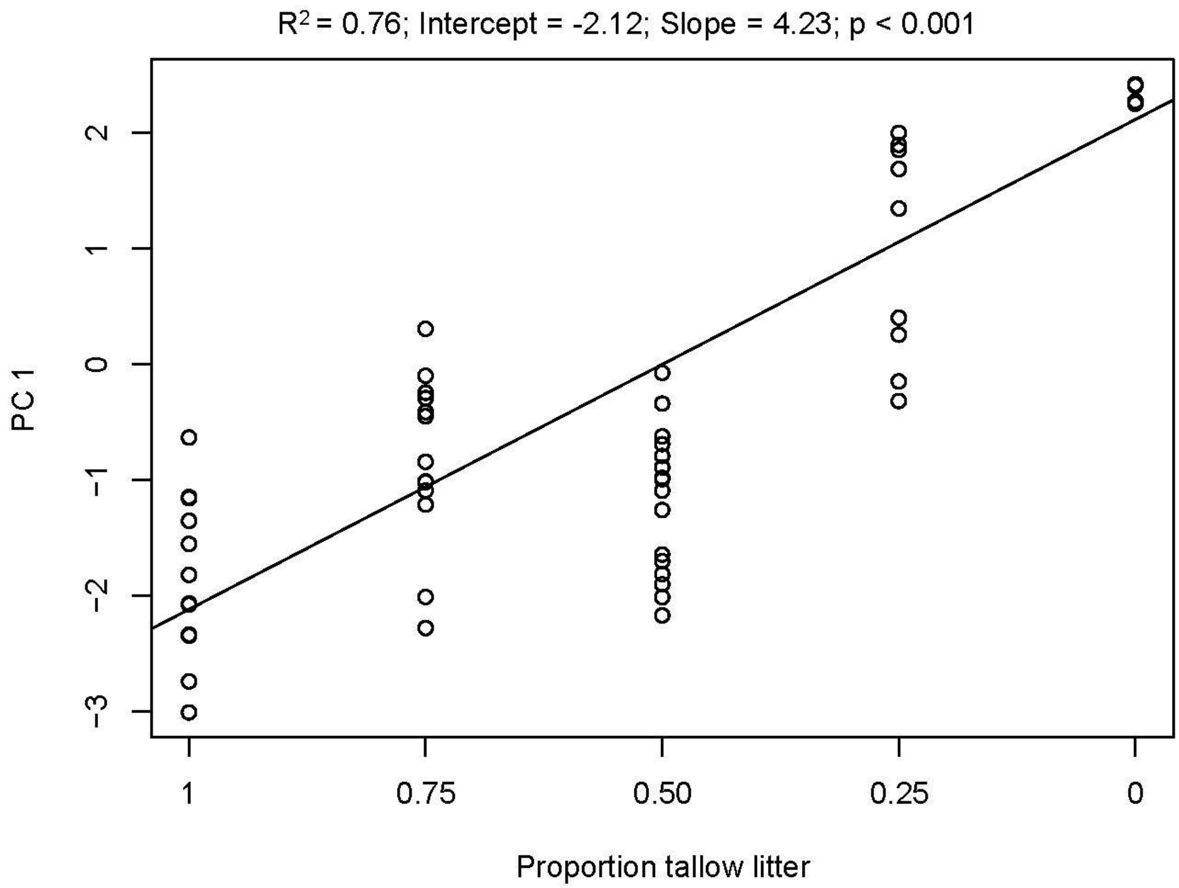

Figure 2-5. Relationship of proportion tallow litter and principal component 1 comprised of combined fire behavior traits (flame height, area burned, rate of spread, and temperature) of the principal component analysis (PCA) measured during experimental burns of mixed pine and tallow at Mary Olive Thomas Demonstration Forest, Auburn, AL, USA.

Seed viability

Post burn seed viability results indicate similar viability regardless of fuelbed proportion of tallow litter ($p = 0.345$) or seed bag treatment ($p = 0.503$; Table 2-2). Similarly, fire temperature did not affect seed viability ($p = 0.410$). For each 25% increase in tallow litter contribution to the fuelbed, we found a 9.68% decrease in seeds with at least 50% char to the seedcoat ($R^2 = 0.29$, $p < 0.0001$); however, char did not significantly impact seed viability ($p = 0.201$). An additional sample of tallow seeds were burned at temperatures between 400 – 1,000 °C and found that for every 100 °C increase in fire temperature, tallow seed viability was reduced by 14.67% (+/- 9.46; +/- 95% CI; $R^2 = 0.46$; $p = 0.005$; Figure 2-6).

Table 2-2. Mean (sd) burn tallow (*Triadica sebifera*) proportion seed viability (PV) and proportion seeds with > 50% char (PC) to the seedcoat for seed exposed to pre-burn fuelbed only (FB), a combination of fuelbed and fire (FBF), and fire only (F) treatment as well as proportion tallow litter contribution to the fuelbed.

	Seed treatment					
	FB		FBF		F	
Prop tallow	PV	PC	PV	PC	PV	PC
0	0.66 (0.13)	0	0.69 (0.13)	0.81 (0.13)	0.64 (0.12)	0.57 (0.24)
0.25	0.64 (0.13)	0	0.65 (0.13)	0.53 (0.32)	0.68 (0.13)	0.46 (0.25)
0.50	0.63 (0.05)	0	0.69 (0.07)	0.54 (0.19)	0.70 (0.11)	0.48 (0.25)
0.75	0.68 (0.14)	0	0.65 (0.08)	0.08 (0.17)	0.66 (0.13)	0.03 (0.06)
1	0.64 (0.15)	0	0.59 (0.16)	0	0.70 (0.04)	0

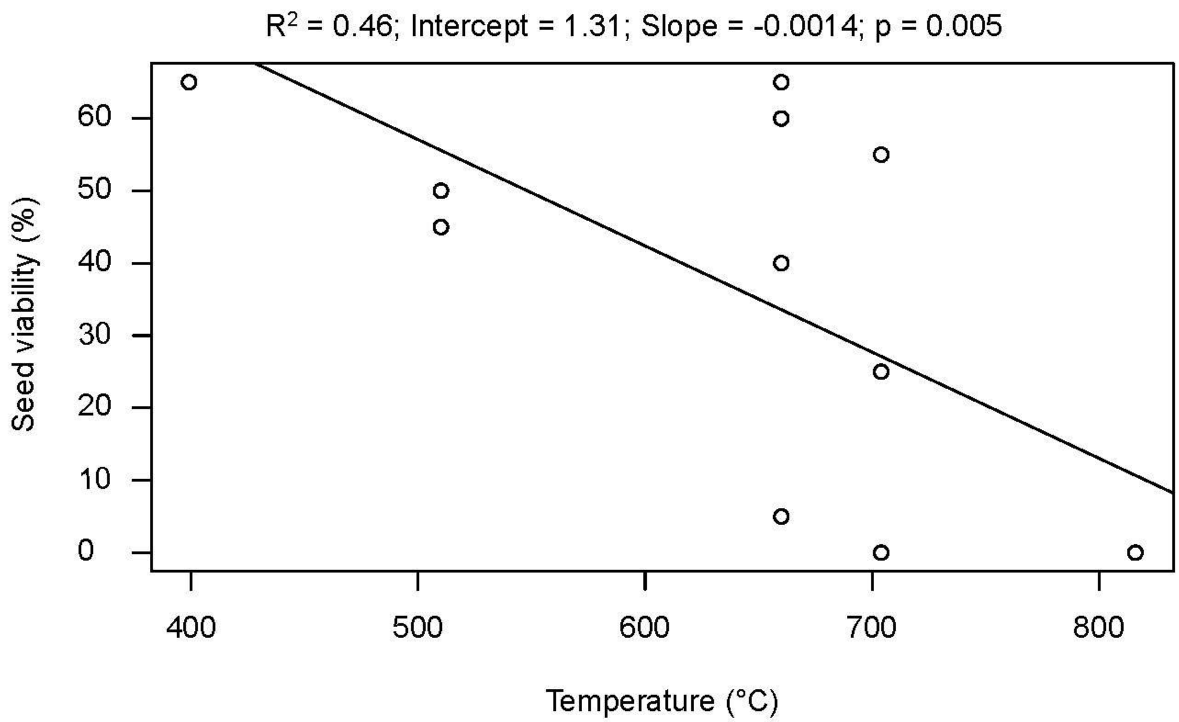

Figure 2-6. Relationship of seed viability to maximum fire temperature for a subsample of tallow (*Triadica sebifera*) seeds burned at Mary Olive Thomas Demonstration Forest in Auburn, Alabama, USA.

Discussion

Experimental Burns

Our study found that increasing proportions of tallow litter contribution to the fuelbed has a negative linear relationship to the rate of spread (ROS), fire temperature, flame height, and area burned. These findings suggest that as the amount of tallow litter in the fuelbed increases, forest flammability will decrease. Our results demonstrate an inflection point between 50 – 75% tallow contribution to the fuelbed where forest flammability declines rapidly. This is consistent with other findings showing fire suppressing behavior when encroaching mesophytic species contribution to the fuelbed is between 33 – 66% (Kreye et al., 2018; McDaniel et al., 2021). While our study divided treatments up in 25% intervals of encroaching litter, other studies used 33%; however, our results still overlap with this fire suppressing range. Tallow litter holds high moisture and forms dense mats on the forest floor (Cameron & LaPoint, 1978; Cameron & Spencer, 1989; Zhang et al., 2017). Similarly, thinner flat leaves typically pack more tightly in the fuelbed and hold higher moisture (Schwilk & Caprio, 2011; Kreye et al., 2013). Our measurements of tallow leaf litter traits show they are thin, lightweight leaves with low curl similar to sweetgum (*Liquidambar styraciflua*), sugar maple (*Acer saccharum*), or red maple (*A. rubrum*) (Table 2-3). Given the similar leaf litter traits between tallow and other fire suppressing species, as well as similar reductions in ROS, fire temperature, flame height, and area burned (McDaniel et al., 2021), we suspect that tallow is capable of transforming ecosystems to promote its growth and spread through the accumulation of less flammable litter in the fuel bed.

Mesophytes are described in Nowacki & Abrams (2008) and expanded upon in Alexander et al. (2021) as species that create shaded, moister, and less flammable forest conditions. Such species as red maple (*Acer rubrum*) and black cherry (*Prunus serotina*) are capable of transforming ecosystems to less flammable states and ultimately replacing the natural, fire-dependent overstory of pines. Given our findings, tallow may use a similar mechanism to facilitate its spread. Increasing tallow litter contribution to the fuelbed has the potential to reduce forest flammability in fire-dependent pine flatwoods and savannas to the detriment of the overstory longleaf and slash pine (*Pinus palustris*, *P. elliottii*).

For this study, we created conditions of tallow and pine at fixed loading conditions to assess the sole impact of tallow contribution to the fuelbed on forest flammability, but under field conditions, the outcome of these burns could be very different. We acknowledge that in real world conditions, other fuel types, fuel loading, and unique weather may be present that impact fire behavior. Additionally, our experimental burns took place in 4-m^2 plots that do not necessarily reflect the heterogeneity of natural fuels.

Seed viability

While some studies have looked at the effect of fire on tallow seedling emergence and germination rate, there is not currently information specifically addressing the impact of fire on tallow seed viability. Under the conditions created during the plot level burns, we did not detect changes in viability in different proportions of tallow litter, however, the number of charred seeds was different. This suggests that while the seeds may not have reached temperatures that negatively impacted seed viability, they may have experienced temperature oscillations that could facilitate germination. Other studies have observed increased germination following fire, likely due to temperature fluctuations (Donahue et al., 2004; Nijjer, 2002; Fan et al., 2021).

Our results did show declines in seed viability for the subsample of tallow seeds burned at extreme temperatures. This is in line with observations from the *Chapter 1* where seedling emergence was 2.04 times higher in unburned plots. Similarly Burns & Miller, 2004 found 7.4

times higher germination of tallow seeds in unburned rather than burned areas. This emphasizes the importance of prescribed fire in the management of tallow in fire-dependent ecosystems. Since increased fire temperature is linked with increased seed mortality (Auld & Denham, 2006; Sabiiti & Wein, 1987; Tangney et al., 2020), we suspect that the decline in viability from high fire temperatures is related to the observed decline in germination and seedling establishment in other studies. While we did not reach high enough temperatures to damage seeds in these plots, this could be different in large scale burns under natural conditions. Even though we did assess the impact of fire on tallow seed viability, one limitation of our study is that we did not investigate if germination differed among burned or unburned seeds in plot level burns, as temperature fluctuations have been shown to promote tallow germination (Donahue et al., 2004).

Table 2-3. Mean (sd) tallow (CT, *Triadica sebifera*) leaf litter morphological traits from a subsample of tallow leaves collected from southern Mississippi in December 2022 compared to values for the following known or suspected mesophytic species: red maple (RM, *Acer rubrum*), sugar maple (SM, *A. saccharum*), and sweetgum (SG, *Liquidambar styraciflua*).

Species	Surface area (cm^2)	Perimeter (cm)	Thickness (mm)	Mass (g)	SLA (cm^2)	Curl (cm)
CT	14.4 (3.4)	22.6 (2.63)	0.14 (0.05)	0.13 (0.04)	165.6 (50.6)	1.4 (0.4)
SG[1]	40.0 (3.0)	40.0 (3.0)	0.15 (0.01)	0.29 (0.03)	183.98 (12.2)	2.9 (0.2)
RM[2]	36.3 (2.0)	*	0.08 (0.00)	*	164.1 (4.8)	1.9 (0.1)
SM[2]	35.0 (2.0)	*	0.06 (0.00)	*	221.4 (8.9)	2.3 (0.1)

[1]McDaniel et al., 2021; [2]Babl et al., 2020; *Data not available

Conclusion

Our findings suggest that Chinese tallow can reduce forest flammability through inputs of litter that are slow to decompose and holds high amounts of moisture. Tallow's ability to transform ecosystems to a less flammable state poses a threat to those that rely on frequent fire to maintain their health and integrity; however, forest flammability is not drastically reduced until a high proportion (e.g., > 50%) of tallow litter is contributed to the fuelbed. These findings suggest that despite potential fire suppressing traits associated with tallow litter, fuelbed conditions are unlikely to exist with high enough proportions of tallow to negatively impact the spread of fire. Additionally, we found no significant differences in tallow seed viability regardless of treatment (F, FB, FBF), proportion tallow, or fire temperature, suggesting that tallow seeds are largely resistant to damage from prescribed fires in invaded ecosystems. Since tallow often invades ecosystems that require frequent fire (e.g., slash and longleaf pine savannas and flatwoods), it could be actively transforming forest flammability to favor its own grown in these fire-dependent ecosystems. Management strategies that focus on the removal mature tallow trees that input low flammability litter should be a top priority to ensure the effectiveness of fire as a management tool is not reduced.

References

Alexander, H. D., Siegert, C., Brewer, J. S., Kreye, J., Lashley, M. A., McDaniel, J. K., Paulson, A. K., Renninger, H. J., & Varner, J. M. (2021). Mesophication of Oak Landscapes: Evidence, Knowledge Gaps, and Future Research. *BioScience*, *71*(5), 531–542. https://doi.org/10.1093/biosci/biaa169

Auld, T. D., & Denham, A. J. (2006). How much seed remains in the soil after a fire? *Plant Ecology*, *187*(1), 15–24. https://doi.org/10.1007/s11258-006-9129-0

Babl, E., Alexander, H. D., Siegert, C. M., & Willis, J. L. (2020). Could canopy, bark, and leaf litter traits of encroaching non-oak species influence future flammability of upland oak forests? *Forest Ecology and Management*, *458*, 117731. https://doi.org/10.1016/j.foreco.2019.117731

Cabrera, S., Alexander, H. D., Willis, J. L., & Anderson, C. J. (2023). Midstory removal of encroaching species has minimal impacts on fuels and fire behavior regardless of burn season in a degraded pine-oak mixture. *Forest Ecology and Management*, *544*, 121157. https://doi.org/10.1016/j.foreco.2023.121157

Cameron, G. N., & Spencer, S. R. (1989). Rapid leaf decay and nutrient release in a chinese tallow forest. *Oecologia*, *80*(2), 222–228. https://doi.org/10.1007/BF00380155

Cameron, G. N., & LaPoint, T. W. (1978). Effects of tannins on the decomposition of Chinese tallow leaves by terrestrial and aquatic invertebrates. *Oecologia*, *32*(3), 349–366. https://doi.org/10.1007/BF00345113

Conn, J. S., Beattie, K. L., & Blanchard, A. (2006). Seed viability and dormancy of 17 weed species after 19.7 years of burial in Alaska. *Weed Science*, *54*(3), 464–470. https://doi.org/10.1614/WS-05-161R.1

Cornwell, W. K., Elvira, A., van Kempen, L., van Logtestijn, R. S. P., Aptroot, A., & Cornelissen, J. H. C. (2015). Flammability across the gymnosperm phylogeny: The importance of litter particle size. *The New Phytologist*, *206*(2), 672–681. https://doi.org/10.1111/nph.13317

Donahue, C., Rogers, W. E., & Siemann, E. (2004). Effects of temperature and mulch depth on Chinese tallow tree (Sapium sebiferum) seed germination. *Texas Journal of Science*, *56*, 347–356.

Hibdon, Lisa M. 2005. Litter dynamics and detrital food webs of native and Chinese tallow stands in a Louisiana bottomland hardwood forest. New Orleans, LA: University of New Orleans. 85 p.

Fan, Z., Song, A., Dong, L., Alexander, H. D., Yang, S., Cheng, N., & Pitchford, J. L. (2021a). Fire effects on post-invasion spread of Chinese tallow (Triadica sebifera) in wet pine flatwood ecosystems in the southeastern United States. *Forest Ecology and Management*, *500*, 119658. https://doi.org/10.1016/j.foreco.2021.119658

Fan, Z., Yang, S., Cheng, N., Liu, X., Song, A., & Dong, L. (2021b). Invasibility of fire-managed ecosystems to the Chinese tallow tree (Triadica sebifera) in the lower Gulf Coastal Plain, USA: Mechanisms and key factors at the landscape level. *Forest Ecology and Management*, *479*, 118539. https://doi.org/10.1016/j.foreco.2020.118539

Fan, Z. (2018). Spatial Analyses of Invasion Patterns of Chinese Tallow (Triadica sebifera) in a Wet Slash Pine (Pinus elliottii) Flatwood in the Coastal Plain of Mississippi, USA. *Forest Science*, *64*(5), 555–563. https://doi.org/10.1093/forsci/fxy014

Grace, J. B. (1998). Can prescribed fire save the endangered coastal prairie ecosystem from the Chinese Tallow invasion? In *Endangered Species UPDATE* (Vol. 15, Issue 5, p. 7076).

Groeschl, D. A., Johnson, J. E., & Smith, D. W. (1993). Wildfire Effects on Forest Floor and Surface Soil in a Table Mountain Pine-Pitch Pine Forest. *International Journal of Wildland Fire*, *3*(3), 149–154. https://doi.org/10.1071/wf9930149

Iverson, L. R., Yaussy, D. A., Rebbeck, J., Hutchinson, T. F., Long, R. P., Prasad, A. M., Iverson, L. R., Yaussy, D. A., Rebbeck, J., Hutchinson, T. F., Long, R. P., & Prasad, A. M. (2004). A comparison of thermocouples and temperature paints to monitor spatial and temporal characteristics of landscape-scale prescribed fires*. *International Journal of Wildland Fire*, *13*(3), 311–322. https://doi.org/10.1071/WF03063

Kreye, J. K., Kane, J. M., & Varner, J. M. (2023). Multivariate roles of litter traits on moisture and flammability of temperate northeastern North American tree species. *Fire Ecology*, *19*(1), 21. https://doi.org/10.1186/s42408-023-00176-5

Kreye, J. K., Varner, J. M., Hamby, G. W., & Kane, J. M. (2018). Mesophytic litter dampens flammability in fire-excluded pyrophytic oak–hickory woodlands. *Ecosphere*, *9*(1), e02078. https://doi.org/10.1002/ecs2.2078

McDaniel, J. K., Alexander, H. D., Siegert, C. M., & Lashley, M. A. (2021). Shifting tree species composition of upland oak forests alters leaf litter structure, moisture, and flammability. *Forest Ecology and Management*, *482*, 118860. https://doi.org/10.1016/j.foreco.2020.118860

Moore, R.P. (1985) Handbook on Tetrazolium Testing. International Seed Testing Association, Zurich.

Nation, R. E., Alexander, H. D., Denny, G., McDaniel, J. K., & Paulson, A. K. (2021). Impacts of increasing fine fuel loads on acorn germination and early growth of oak seedlings. *Fire Ecology*, *17*(1), 1. https://doi.org/10.1186/s42408-020-00088-8

Nijjer, S., Lankau, R., Rogers, W. E., & Siemann, E. (2002). Effects of temperature and light on Chinese tallow (Sapium sebiferum) and Texas sugarberry (Celtis laevigata) seed germination. *Texas Journal of Science*, *54*, 63–68.

NOAA NCEI U.S. Climate Normals Quick Access. (n.d.). Retrieved October 4, 2021, from https://www.ncei.noaa.gov/access/us-climate-normals/#dataset=normals-annualseasonal&timeframe=30&location=MS&station=US1MSJC0012

Nowacki, G. J., & Abrams, M. D. (2008). The demise of fire and "mesophication" of forests in the eastern United States. *BioScience. 58(2): 123-138.*, *58*(2), Article 2. https://doi.org/10.1641/b580207

NRCS *Web Soil Survey—Home*. (n.d.). Retrieved October 5, 2021, from https://websoilsurvey.sc.egov.usda.gov/App/HomePage.htm

Pile, L. S., Wang, G. G., Waldrop, T. A., Walker, J. L., Bridges, W. C., & Layton, P. A. (2017). Managing an Established Tree Invader: Developing Control Methods for Chinese Tallow (Triadica sebifera) in Maritime Forests. *Journal of Forestry*, *115*(6), 522–529. https://doi.org/10.5849/JOF-2016-022R2

Pimentel, D., Zuniga, R., & Morrison, D. (2005). Update on the environmental and economic costs associated with alien-invasive species in the United States. *Ecological Economics*, *52*(3), 273–288. https://doi.org/10.1016/j.ecolecon.2004.10.002

Rothermel, R. C. (1972). A mathematical model for predicting fire spread in wildland fuels. *Res. Pap. INT-115. Ogden, UT: U.S. Department of Agriculture, Intermountain Forest and Range Experiment Station. 40 p.*, *115*. https://www.fs.usda.gov/research/treesearch/32533

R Core Team (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Renne, I. J., Spira, T. P., & Bridges, W. C. (2001). Effects of Habitat, Burial, Age and Passage through Birds on Germination and Establishment of Chinese Tallow Tree in Coastal South Carolina. *The Journal of the Torrey Botanical Society*, *128*(2), 109–119. https://doi.org/10.2307/3088733

Renne, I. J., Barrow, W. C., Randall, L. A. J., & Bridges, W. C. (2002). Generalized avian dispersal syndrome contributes to Chinese tallow tree (Sapium sebiferum, Euphorbiaceae) invasiveness. *Diversity and Distributions*, *8*(5), 285–295. https://doi.org/10.1046/j.1472-4642.2002.00150.x

Sabiiti, E. N., & Wein, R. W. (1987). Fire and Acacia Seeds: A Hypothesis of Colonization Success. *Journal of Ecology*, *75*(4), 937–946. https://doi.org/10.2307/2260305

Schwilk D.W. , A.C. Caprio. *Scaling from leaf traits to fire behaviour: Community composition predicts fire severity in a temperate forest—Schwilk—2011—Journal of Ecology—Wiley Online Library*. (n.d.). Retrieved May 17, 2023, from https://besjournals.onlinelibrary.wiley.com/doi/full/10.1111/j.1365-2745.2011.01828.x

Susko, D., Mueller, J., & Spears, J. (2011). An evaluation of methods for breaking seed dormancy in kudzu (Pueraria lobata). *Canadian Journal of Botany*, *79*, 197–203. https://doi.org/10.1139/b00-153

Tangney, R., Merritt, D. J., Callow, J. N., Fontaine, J. B., & Miller, B. P. (2020). Seed traits determine species' responses to fire under varying soil heating scenarios. *Functional Ecology*, *34*(9), 1967–1978. https://doi.org/10.1111/1365-2435.13623

Varner, J. M., Kane, J. M., Kreye, J. K., & Engber, E. (2015). The Flammability of Forest and Woodland Litter: A Synthesis. *Current Forestry Reports*, *1*(2), 91–99. https://doi.org/10.1007/s40725-015-0012-x

Web Soil Survey—Home. (n.d.). Retrieved October 5, 2021, from https://websoilsurvey.sc.egov.usda.gov/App/HomePage.htm

Zhang, L., Zou, J., & Siemann, E. (2017). Interactive effects of elevated CO2 and nitrogen deposition accelerate litter decomposition cycles of invasive tree (Triadica sebifera). *Forest Ecology and Management*, *385*, 189–197. https://doi.org/10.1016/j.foreco.2016.11.045

Chapter 3: Chinese tallow (*Triadica sebifera*) resprouting vigor and belowground nonstructural carbohydrate stores in slash and longleaf pine (*Pinus elliottii; P. palustris*) flatwoods and savannas along the Gulf of Mexico Coast

Abstract

Endangered coastal pine flatwoods and savannas found in the southeastern US consisting of an overstory of longleaf and slash pine (*Pinus palustris, P. elliottii*, respectively) are at risk of invasion from Chinese tallow *(Triadica sebifera*), an invasive exotic tree resilient to fire and other disturbances. While tallow is susceptible to topkill by prescribed fire often used in managing these coastal ecosystems, it can quickly resprout from the stem, fostering tallow persistence and spread in these systems. This study aimed to characterize the post-topkill mortality, resprouting vigor (aggregate resprout height), and belowground nonstructural carbohydrate stores of tallow trees at two sites located in southern Mississippi, USA with different distance from the Gulf of Mexico (GOM): Grand Bay National Estuarine Research Reserve (GB; 2 km distance) and Mississippi Sandhill Crane National Wildlife Refuge (SH; 9 km distance). At each site, we selected a group of tallow trees of varying sizes and age classes and topkilled them using saws to mimic fire-induced topkill. We then monitored their survival and resprouting vigor after one year. In addition to topkilling trees, we collected seedlings ranging from 2-4 years old to assess differences in belowground nonstructural carbohydrate stores that could potentially influence mortality and resprouting vigor following topkill in young trees. We measured environmental conditions such as canopy closure and soil electric conductivity to characterize potential site differences that could help explain our findings. We found that initial resprouting vigor in tallow trees is driven by canopy closure and tree size, with larger trees under open canopies resprouting with the greatest aggregate resprout height. Total

belowground nonstructural carbohydrates were similar between sites; however, root starch (%) was ~ 2 times higher at SH, while sugar was ~ 1.5 times higher at GB. Additionally, seedlings at GB had ~ 7 cm shorter roots but ~ 1 g greater root biomass, suggesting a limited rooting zone and nutrient-poor growing conditions that could adversely affect survival. While the scope of these findings is limited to two study sites in southern Mississippi, USA, the range of responses exhibited by tallow across sites suggests some environmental factors could limit the invasibility of at-risk ecosystems close to the coast. Similarly, management and removal of invasive tallow in certain sites, specifically with prescribed fire, could be more effective in controlling this species by limiting resprouting after topkill.

Introduction

Despite efforts to maintain coastal longleaf and slash pine (*Pinus palustris* and *P. elliottii*, respectively) flatwoods and savannas, invasive species remain prevalent in these ecosystems. In the southeast United States, Chinese tallow tree (*Triadica sebifera*; hereafter: tallow) is an invasive exotic species that is expected to continue expanding its range due to prolific seed production with a long dispersal range via birds and water currents (Grace, 2001; Lin et al., 1958; Renne et al., 2002). The overstory trees offer ideal perches for birds which aid in the distribution and spread of tallow via zoochory (Fan 2018, Fan et al., 2021; Renne et al., 2001). Due to the increased risk of invasion faced by coastal pine flatwoods and savannas and the high degree of biodiversity they support, it is critical to understand how tallow interacts with management strategies such as prescribed fire to effectively control this species' spread.

In fire-dependent coastal forests, prescribed fire is a frequently used tool to maintain the structure and diversity of these ecosystems (U.S. Fish and Wildlife Service, 2010); however, the use of prescribed fire can facilitate the spread of tallow through basal sprouting (Grace, 1998; Grace et al., 2005). Resprout vigor is defined as the aggregate height of resprouts, which takes in account both the number and length of resprouts on a tree (Fei et al., 2006). Tallow is capable of vigorously resprouting following topkill from disturbances such as fire (Grace, 1998; Scheld and Cowels, 1981), and can resprout in as little as 1-month after being topkilled (Miller, 2003). Resprouts can grow > 3 m in as little as one year and up to 8 m in two years (Rockwood and Geary, 1991; Scheld and Cowels, 1981), and some topkilled tallow trees demonstrated a 37% survival rate after three years (Rockwood and Geary, 1991). Additionally, the sprouts from developed root systems are capable of dramatically altering species composition post-disturbance by outcompeting other species that resprout less vigorously (Keyser and Zarnoch 2014), potentially further facilitating the spread of invasive species by shading out other less competitive species. While herbicide treatment can prove helpful for limiting tallow invasion (Bruce et al., 1997), some areas cannot use this technique due to the sensitive wildlife species on wildlife refuges and federal lands. As such, the preferred method for removing tallow without herbicide treatment involves repeated prescribed fire and the manual felling of trees on site (Bruce et al., 1997); however, given the ability of tallow to resprout, these techniques can instead facilitate further invasion by numerous resprouts that quickly reach reproductive maturity (Jubinsky, 1995).

One mechanism that may facilitate tallow's resprouting capacity is the species' ability to store carbohydrates belowground. Root starch stores are critical to a tree's ability to resprout (Miyanishi and Kellman, 1986; von Fircks & Sennerby-Forsse, 1998). The time of year influences the amount of nonstructural carbohydrates stored in tallow roots and was lowest during seed production and highest prior to leaf fall in tallow (Conway et al., 1999). Additionally, resprouting vigor following the removal of aboveground biomass is largely determined by belowground nonstructural carbohydrates stored in the roots as well as available soil nutrients such as nitrogen (Kobe, 1997; Cruz et al., 2003; Vesk & Westoby, 2004), individual tree size, and number of latent buds (Lloret & Lopez-Soria, 1993; Canadell & Lopez-Scoria, 1998). Storage of belowground carbohydrates is critical to the regeneration of aboveground biomass following topkill, and as a result, could impact the capacity of species such as tallow to regenerate following disturbance.

Environmental conditions, along with tallow's belowground carbohydrate storage, may also impact the likelihood of resprouting. Tallow growing in saturated conditions was successfully killed if the trees were cut below the water level (Bruce et al., 1997). While tallow seedlings can survive in soils saturated with high salinity water between 20 – 27 ppt (~ 32 - 42 mS/cm EC), these trees showed reduced belowground biomass compared to tallow seedlings growing in non-saline soils. (Conner, 1994, Conner and Askew, 1993). Similarly, along a salinity gradient in southern Mississippi, soil salinity levels > 2 mS/cm lowered the establishment of tallow seedlings, suggesting that salinity may play an important role in regulating the growth and establishment of native and exotic species in coastal environments (Paudel and Battaglia, 2015). Despite tallow being shade-tolerant, light availability is an important factor in its ability to successfully resprout (Cameron et al., 2000; Lin et al., 2004; Meyer, 2011). These findings suggest that on-site environmental conditions such as soil moisture, soil salinity, and canopy cover may play a role in regulating the spread and proliferation of tallow.

For this study, we had two primary objectives: 1) better understand how tallow seedling age and size influence belowground nonstructural carbohydrate stores at two sites located different distances (< 2 km and ~9 km) from the Gulf of Mexico (GOM), and 2) assess whether tree size, canopy closure, and site impact tallow mortality and resprout vigor (aggregate resprout height) following topkill. We hypothesized that regardless of seedling age, tallow seedlings growing at the site closest to the coast would have a lower root:shoot ratio, belowground biomass, and non-structural carbohydrate stores, specifically starch, due to increased stress from poorer soil conditions, ultimately leading to decreased ability of tallow to resprout and higher tallow mortality following topkill. Broadly, we expect harsher growing conditions nearest the coast to increase tallow mortality and reduce resprouting vigor following topkill due to lower belowground biomass and carbohydrate stores in the roots, which may facilitate successful management of tallow by using prescribed fire at these sites.

Methods

Study Sites

Research was conducted at two study sites located different distances from the GOM (Table 3-1): Mississippi Sandhill Crane National Wildlife Refuge (SH) (30.475°N, 88.783°W) and Grand Bay National Estuarine Research Reserve and Wildlife Management Area (GB) in Jackson County, Mississippi (30.377°N 88.434°W), USA. Both sites have similar average annual high and low temperatures (26 °C (July) and 12 °C (January), respectively (NOAA, 2021)) and average annual precipitation that peaks in summer between June and August (175 cm and 169 cm, respectively (NOAA, 2021)). SH sits further from the GOM coast (9 km) than SH (< 2 km). Additionally, SH has a higher average elevation of 11 m above sea level compared to GB which sits at or below 2 m for most of its expanse.

Soils at SH are similar to GB but are dominated by very fine sandy loams and loamy alluvium as opposed to poorly drained silty loams and loamy sands (NRCS, 2021; Table 3-1). The USDA-NRCS web soil survey map for GB indicated two primary soil series: Hyde silt loam and Myatt loam, 0 to 1 percent slopes, occasionally flooded. Hyde silt loam is a very poorly drained soil with moderate permeability that makes up most of this study site. Hyde silt loam is a thermic Typic Umbraquults that is very poorly drained. Another soil series, Myatt loam, comprises the rest of this site. This soil is a thermic Typic Endoaquults that is poorly drained and moderately permeable. SH was characterized by one primary soil type outlined by the USDA-NRCS web soil survey map: bayou sandy loam, 0 to 1 percent slopes. The bayou soil series is a thermic Typic Paleaquult with low slope, typically occurring in woodlands. This soil is poorly drained and has moderately slow permeability throughout the soil profile.

Both sites contain flatwood and savanna forests dominated by a sparse canopy of low-density longleaf pine (*Pinus palustris*) and slash pine (*P. elliottii*). These forest types require frequent, low-intensity surface fire every 1 – 3 years to maintain their structure and rich biodiversity. As such, they have been managed with prescribed fire since the late 1970s. Additionally, both sites have a widespread problem with invasive species, including tallow trees. This makes our selected sites ideal for examining tallow's resprouting capacity differences.

Sites also varied in environmental conditions (Table 3-1). Based on a one-time sampling in March 2022, site-wide surface (6-cm depth) electrical conductivity (EC) (a proxy for soil salinity; Dingkuhn & Le Gal, 1996; Asch & Wopereis, 2001; Abdennour et al., 2020) and soil moisture, both measured with a soil probe (model YY-1000, Yieryi Manufacturing, Longgang District, Shenzhen, Guangdong Province, China), were 0.04 mS/cm and 35.0%, respectively, at SH, while at GB, they were 0.28 mS/cm and 37.9%, respectively. Canopy cover was estimated with a spherical densiometer at SH in April 2016 and GB in March 2022, and mean values for SH and GB were 34% and 54%, respectively. Note that these soil condition values from one point in time likely do not capture the true range of soil conditions experienced at these two sites, which not only vary in their distances from the GOM, but also likely in associated conditions like degree of tidal flooding and water table depths.

Table 3-1. Site wide differences between environmental conditions (mean (SD)) at Mississippi Sandhill Crane National Wildlife Refuge (SH) and Grand Bay National Estuarine Research Reserve (GB) in southern Mississippi, USA. Soil salinity and soil moisture are mean (± sd) values from a one-time sampling at the two study sites in March 2022. Canopy cover is mean (± sd) from measurements at GB in March 2022 and SH in April 2016.

Location	Soil salinity (EC; mS/cm)	Soil moisture (%)	Soil type[1]	Canopy cover (%)	Elevation (m)	Distance from Gulf of Mexico (km)
SH	0.04 (0.05)	35.0 (16.3)	Very fine sandy loams loamy alluvium	34 (31.6)	11	9
GB	0.28 (0.21)	37.9 (15.9)	Poorly drained silty loams loamy sands	54 (17.1)	<2	<2

[1]USDA-NRCS Web Soil Survey

Belowground nonstructural carbohydrates

To measure differences in belowground nonstructural carbohydrates that could impact resprouting capacity, we collected 60 tallow seedlings (30 from each site) ranging in age from 2-4 years old (estimated by counting growth rings at the root collar). We collected seedlings from a single 0.01-ha area at each site where tallow seedlings were abundant and forest structure, composition, and canopy closure were similar. Seedlings were collected by carefully loosening the soil around each individual using a dibble bar and then gently pulling from the soil to preserve the root condition.

Seedlings were transported from the sites in a cooler and processed immediately upon return to the lab where we prepared the root samples for further processing by rinsing in tap water to remove all dirt. Seedling total length, root length, and stem length were measured with a meter stick, and root collar diameter (RCD) was measured with calipers before dividing the seedlings in half at the root collar using a knife. These pre-processing measurements were used to calculate a root:shoot ratio based on length for the seedlings. Roots were then microwaved for 90 seconds at 600 watts to stop any cellular activity. Following this, we dried the samples in an oven at 100 °C for one hour and, immediately after, allowed the samples to dry for three days at 70 °C until a constant dry weight. After three days, the roots and tops were weighed to calculate a total biomass and a root:shoot ratio based on biomass for the tallow seedlings. Finally, tallow roots weighing more than 10 g were ground into a fine powder and stored at room temperature before being sent to the RTP Analytical Lab, Raleigh, NC, US for processing that included information on % sugar, % starch, and % tNSC (combined sugar and starch; total nonstructural carbohydrates).

Tallow mortality and resprouting vigor

To assess tallow mortality and resprouting vigor following topkill, we topkilled tallow trees ranging in size from 2.5 – 50 cm diameter at breast height (DBH). Because using fire to topkill tallow trees on site was not possible for our study, as federal lands have strict regulations on who can implement prescribed fires and when, we opted to cut tallow trees with saws or clippers (depending on tree size) above ground level to simulate the hydraulic death of plants caused by fire or other natural disturbances (Hoffmann et al., 2009; Midgley et al., 2011). While mechanical is a different mechanism of topkill than fire, previous work has shown clipping and fire to produce similar resprouting and mortality responses in *Eric multiflora* (Lloret & Lopez-Soria, 1993). At GB, we worked with on-site personnel to manually topkill approximately 200 trees in March 2022. At SH, 176 trees were cut in March 2016 as part of a previous study using similar methods (Poyner, 2017). After cutting, we measured the height of the cut above the ground line and tagged trees with a unique number. At GB, we monitored topkilled trees in May and October 2022 and March and May 2023 to assess any new growth or mortality, while trees at SH were monitored in August and November of 2016 and December 2017. We measured the number of resprouts and the length of resprouts with a tape (precision: +/- 5 cm) to create a combined aggregate height of sprouts used to estimate resprouting vigor (Lloret & Lopez-Soria, 1993; Fei et al., 2006).

At GB, we measured EC and soil moisture at the time of topkill with a soil probe near the base of each cut individual as described above, which were averaged to estimate this site's EC and soil moisture listed in Table 3-1. Our initial goal was to link these measurements with resprouting vigor, but EC values never exceeded values indicative of high salinity (4 mS/cm) typically associated with negative plant establishment and growth (USDA-NRCS, 2011); therefore, we abandoned this approach. At SH, EC and soil moisture were measured in March 2022 at approximately 20 locations within the stand where trees were topkilled and averaged for site-wide values (Table 3-1). In April 2016, the SH study site was subdivided into three plots and canopy cover was averaged from 10 spherical densiometer readings in each plot. At the GB study site, we did not subdivide the plot since canopy cover was similar throughout, and in March 2022, we took 12 densiometer readings within the GB plot to estimate this study site's canopy cover.

Data analysis:

To compare seedling differences between sites, we performed a Student's t test on seedling shoot length, root length, total length, and root collar diameter (RCD). We used an analysis of covariance (ANCOVA) to test for site and seedling age (the covariate taking 2, 3, or 4 years, respectively) effects on the six aspects of tallow seedling roots, including total nonstructural carbohydrates (%), sugar (%), and starch (%) as well as the log-transformed values for a sugar:starch ratio, root:shoot length ratio, and a root:shoot biomass ratio. Before ANCOVA, these variables were tested for normality and equal variance assumptions, and corresponding (e.g., logarithmic) transformations were conducted. We performed an ANCOVA on the log-transformed aggregated resprout height to test the impact of site and DBH on resprouting vigor with canopy closure as a covariate. We employed a logistic regression with the same set of variables to assess their impact on resprout survival. All data analyses were performed in R-4.2.1 with a significance level of $\alpha = 0.05$ (R Core Team, 2021).

Results

Tallow seedlings between 2- and 4-years old were larger at SH than GB (Table 3-2). Seedling shoot length was 9.2 cm (+/- 9.06; +/- 95% CI) greater at SH than GB ($p = 0.047$). Similarly, root length was 6.7 cm (+/- 9.06; +/- 95% CI) greater at SH than GB ($p = 0.009$). Total seedling length followed the same trend, with seedlings at SH being 15.5 cm (+/- 11.49; +/- 95% CI) taller than those at GB ($p = 0.009$). Seedling root collar diameter (RCD) was again greater at SH than GB by 3.7 mm (+/- 1.32; +/- 95% CI; $p < 0.001$).

Total nonstructural carbohydrates (%) were lower at GB ($p = 0.056$; Figure 3-1A). Root sugar (%) was higher at GB ($p <0.001$; Figure 3-1B); however, root starch (%) was lower ($p = 0.007$; Figure 3-1C). Seedlings growing at GB had a higher sugar:starch ratio in their roots than those growing at SH ($p <0.001$; Figure 3-1D). The root:shoot length ratio was lower at GB compared to SH ($p = 0.069$; Figure 3-1E). In contrast, the root:shoot biomass ratio was higher at GB and lower at SH ($p = 0.0352$; Figure 3-1F).

Resprout vigor varied with tree size (DBH; $p < 0.001$) and canopy cover ($p = 0.002$) but not site ($p = 0.196$). Larger trees had a greater resprout vigor, and higher canopy cover led to lower resprout vigor. For the 376 tallow trees topkilled for this study, 13.9% died within 1 year of cutting. Mortality was significantly higher at GB ($p < 0.001$; Figure 3-2). Similar to resprout vigor, tree size ($p = 0.025$) and canopy cover ($p = 0.04$) were inversely related to tallow mortality, with larger trees being more likely to survive and higher canopy cover reducing survival probability.

Table 3-2. Chinese tallow (*Triadica sebifera*) seedling (mean (sd)) shoot length, root length, total length, and root collar diameter (RCD) for Grand Bay National Estuarine Research Reserve (GB) and Mississippi Sandhill Crane National Wildlife Refuge (SH) in southern Mississippi, USA. All response variables were significantly different ($p < 0.05$) between sites.

Location	Shoot length (cm)	Root length (cm)	Total length (cm)	RCD (mm)
GB	62.12 (21.61)	19.65 (17.17)	81.78 (26.14)	7.47 (2.24)
SH	71.25 (30.45)	35.02 (13.85)	97.30 (38.73)	11.15 (2.99)

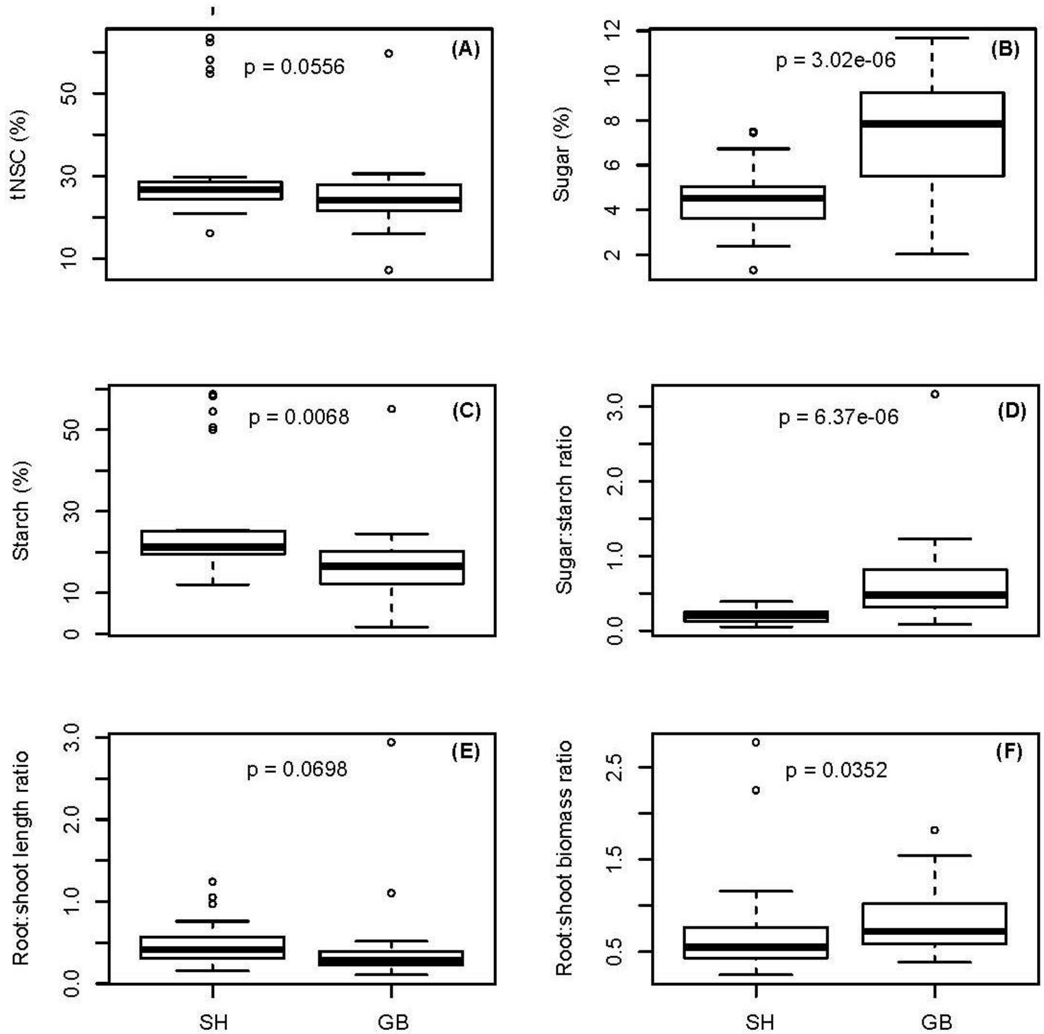

Figure 3-1. Chinese tallow seedling root (A) total nonstructural carbohydrates (tNSC), (B) sugar, (C) starch, (D) sugar:starch ratio, (E) root:shoot length ratio, and (F) root:shoot biomass ratio between Mississippi Sandhill Crane National Wildlife Refuge (SH) and Grand Bay National Estuarine Research Reserve (GB) sites in southern Mississippi, USA.

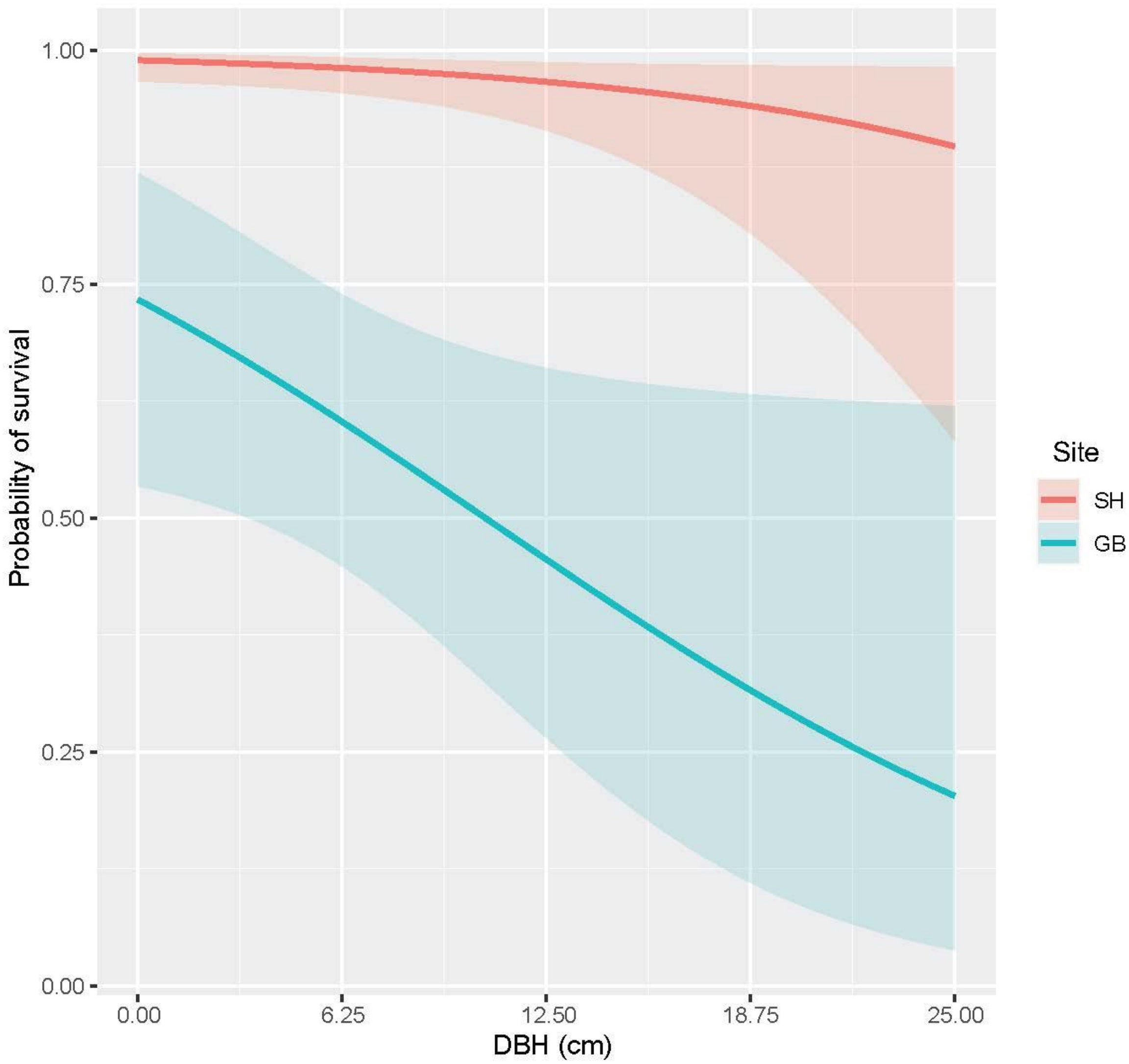

Figure 3-2. Predicted probability of survival following topkill by cutting for Chinese tallow (*Triadica sebifera*) trees by size (DBH, cm) at Mississippi Sandhill Crane National Wildlife Refuge (SH) and Grand Bay National Estuarine Research Reserve (GB) in southern Mississippi, USA.

Discussion

Our findings revealed key differences in tallow ability to resprout following topkill at the two sites. At both sites, tNSC were similar in 2–4-year-old seedlings; however, the ratio of sugar and starch differed. Tallow seedlings growing in GB had greater sugar relative to starch in their roots, suggesting that starches had been utilized to sustain survival and growth in the relatively harsher soil conditions. Because starch is the main source of remobilized carbon during resprouting and growth in species such as aspen (*Populus tremuloides*) (Smith et al., 2018; Wiley et al., 2019), the higher sugar:starch ratio for seedlings growing at GB may explain why trees at this site were less likely to resprout following topkill, and instead, were more likely to die. Furthermore, tallow seedlings growing at GB in a previous study demonstrated reduced root collar diameter, root and shoot length, and root and shoot biomass (Conner, 1994), comparable to our findings of lower seedling size at GB compared to SH.

We found that canopy cover also influenced tallow mortality following topkill. Trees growing in areas with high canopy cover were less likely to survive and resprout than those in locations with low canopy cover. This finding is supported by other studies that show tallow trees grow faster and have a higher survival rate in open areas with high sunlight exposure (Cameron et al., 2000; Meyer, 2011). Lin et al. (2004) found that tallow seedlings growing in low light conditions had the highest mortality risk. Siemann & Rogers (2003) also found a similar trend, with tallow seedlings growing in low light areas experiencing lower survival than those in open canopy conditions with high light. Given these results and the two primary ecosystems of concern in the region, the open, sparse canopy of pine savannas are likely at an increased risk of invasion due to the limited canopy closure. The open canopy paired with tree perches that facilitate seed dispersal by birds (Fan, 2018; Fan et al., 2021b; Renne et al., 2001), make them vulnerable to invasion by tallow.

Our results demonstrate an increasing aggregate resprout height with increasing tree size that coincides with findings from other studies (Matula et al., 2019; Vesk, 2006). This could be due to the higher nonstructural carbohydrate stores we observed in larger trees, or that larger trees have the potential to have more latent buds that are linked to resprout success (Lloret & Lopez-Soria, 1993; Canadell & Lopez-Scoria, 1998; Vesk & Westoby, 2004). Because the larger tallow trees are more likely to survive and resprout, additional precautions should be taken to ensure they do not resprout following intervention.

Our initial attempt was to investigate the potential role of EC on tallow mortality and resprout vigor; however, our measured range between the two sites was very narrow and did support a suitable analysis of this factor (Table 3-2). Soil EC and salinity have been shown to be important factors on tallow growth (Conner & Askew, 1993; Conner, 1994; Paudel and Battaglia 2015). Still, other soil factors could be driving the site-specific differences in mortality and resprout vigor we observed in this study. Beyond soil EC, soil drainage class and rooting zone could play an important role in tallow mortality and nonstructural carbohydrate allocation. Because tallow seedlings at GB had greater belowground biomass, but shorter overall root length, this suggests that the soils may be nutrient poor and difficult for tallow to grow in. Nutrient-poor soils with a low cation exchange capacity (CEC) often result in plants needing increased root system size to support the plant (Grier, 1981; Cavelier, 1992). This is reflected in the higher seedling root:shoot ratio we found at GB compared to seedlings at SH.

This study provides valuable baseline data of tallow response to being topkilled and it is important to take into consideration other biological and environmental factors that could also drive the observed differences in tallow mortality and resprouting vigor at our study sites. Still, our findings are relevant for the management of fire-dependent ecosystems that are threatened by tallow invasion since the effectiveness of fire in controlling the spread of tallow could be compromised at sites further from the coast.

References

Abdennour, M. A., Douaoui, A., Piccini, C., Pulido, M., Bennacer, A., Bradaï, A., Barrena, J., & Yahiaoui, I. (2020). Predictive mapping of soil electrical conductivity as a Proxy of soil salinity in south-east of Algeria. *Environmental and Sustainability Indicators*, *8*, 100087. https://doi.org/10.1016/j.indic.2020.100087

Asch, F., & Wopereis, M. C. S. (2001). Responses of field-grown irrigated rice cultivars to varying levels of floodwater salinity in a semi-arid environment. *Field Crops Research*, *70*(2), 127–137. https://doi.org/10.1016/S0378-4290(01)00128-9

Barrilleaux, T. C., & Grace, J. B. (2000). Growth and Invasive Potential of Sapium sebiferum (Euphorbiaceae) within the Coastal Prairie Region: The Effects of Soil and Moisture Regime. *American Journal of Botany*, *87*(8), 1099–1106. https://doi.org/10.2307/2656646

Bruce, Katherine A.; Cameron, Guy N.; Harcombe, Paul A.; Jubinsky, Greg. 1997. Introduction, impact on native habitats, and management of a woody invader, the Chinese tallow tree, Sapium sebiferum (L.) Roxb. *Natural Areas Journal*. 17(3): 255-260.

Bruce, Katherine A. 1993. Factors affecting the biological invasion of the exotic Chinese tallow tree, Sapium sebiferum, in the Gulf Coast prairie of Texas. Houston, TX: University of Houston. 155 p. Thesis.

Cameron, G. N., & Spencer, S. R. (1989). Rapid leaf decay and nutrient release in a Chinese tallow forest. *Oecologia*, *80*(2), 222–228. https://doi.org/10.1007/BF00380155

Cameron, G. N., Glumac, E. G., & Eshelman, B. D. (2000). Germination and Dormancy in Seeds of Sapium sebiferum (Chinese Tallow Tree). *Journal of Coastal Research*, *16*(2), 391–395.

Canadell, J., & López-Soria, L. (1998). Lignotuber reserves support regrowth following clipping of two Mediterranean shrubs. *Functional Ecology*, *12*(1), 31–38. https://doi.org/10.1046/j.1365-2435.1998.00154.x

Casals, P., & Rios, A. I. (2018). Burning intensity and low light availability reduce resprouting ability and vigor of Buxus sempervirens L. after clearing. *Science of The Total Environment*, *627*, 403–416. https://doi.org/10.1016/j.scitotenv.2018.01.227

Cavelier, J. (1992). Fine-root biomass and soil properties in a semideciduous and a lower montane rain forest in Panama. *Plant and Soil*, *142*(2), 187–201. https://doi.org/10.1007/BF00010965

Conner, W. H., & Askew, G. R. (1993). Impact of Saltwater Flooding on Red Maple, Redbay, and Chinese Tallow Seedlings. *Castanea*, *58*(3), 214–219.

Conner, W. H. (1994). The Effect of Salinity and Waterlogging on Growth and Survival of Baldcypress and Chinese Tallow Seedlings. *Journal of Coastal Research*, *10*(4), 1045–1049.

Conway, W. C., Smith, L. M., Sosebee, R. E., & Bergan, J. F. (1999). Total Nonstructural Carbohydrate Trends in Chinese Tallow Roots. *Journal of Range Management*, *52*(5), 539. https://doi.org/10.2307/4003784

Cruz, A., Pérez, B., & Moreno, J. M. (2003). Plant stored reserves do not drive resprouting of the lignotuberous shrub Erica australis. *New Phytologist*, *157*(2), 251–261. https://doi.org/10.1046/j.1469-8137.2003.00668.x

Dingkuhn, M., & Le Gal, P.-Y. (1996). Effect of drainage date on yield and dry matter partitioning in irrigated rice. *Field Crops Research*, *46*(1), 117–126. https://doi.org/10.1016/0378-4290(95)00094-1

Erickson, H. E., & White, R. (2007). *Invasive plant species and the Joint Fire Science Program.* (PNW-GTR-707; p. PNW-GTR-707). U.S. Department of Agriculture, Forest Service, Pacific Northwest Research Station. https://doi.org/10.2737/PNW-GTR-707

Fan, Z., Song, A., Dong, L., Alexander, H. D., Yang, S., Cheng, N., & Pitchford, J. L. (2021a). Fire effects on post-invasion spread of Chinese tallow (Triadica sebifera) in wet pine flatwood ecosystems in the southeastern United States. *Forest Ecology and Management*, *500*, 119658. https://doi.org/10.1016/j.foreco.2021.119658

Fan, Z., Yang, S., Cheng, N., Liu, X., Song, A., & Dong, L. (2021b). Invasibility of fire-managed ecosystems to the Chinese tallow tree (Triadica sebifera) in the lower Gulf Coastal Plain, USA: Mechanisms and key factors at the landscape level. *Forest Ecology and Management*, *479*, 118539.

Fan, Z., Yang, S., Loewenstein, N. J., Cheng, N., Nepal, S., Pitchford, J. L., Chappell, J., & Stone, D. (2021c). Modeling spatial variations of the invasibility of slash pine flatwoods to Chinese tallow (Triadica sebifera) invasion: Mechanisms and key factors at the microscale. *Forest Ecology and Management*, *482*, 118798. https://doi.org/10.1016/j.foreco.2020.118798

Fan, Z., Yang, S., & Liu, X. (2018). Spatiotemporal Patterns and Mechanisms of Chinese Tallowtree (Triadica sebifera) Spread along Edge Habitat in a Coastal Landscape, Mississippi, USA. *Invasive Plant Science and Management*, *11*(3), 117–126. https://doi.org/10.1017/inp.2018.21

Fan, Z. (2018). Spatial Analyses of Invasion Patterns of Chinese Tallow (Triadica sebifera) in a Wet Slash Pine (Pinus elliottii) Flatwood in the Coastal Plain of Mississippi, USA. *Forest Science*, *64*(5), 555–563. https://doi.org/10.1093/forsci/fxy014

Fei, S., Gould, P. J., Steiner, K. C., & Finley, J. C. (2006). Aggregate height—A composite measure of stand density for tree seedling populations. *Forest Ecology and Management*, *223*(1), 336–341. https://doi.org/10.1016/j.foreco.2005.11.014

Flory, S. L., Dillon, W., & Hiatt, D. (n.d.). Interacting global change drivers suppress a foundation tree species. *Ecology Letters*, *n/a*(n/a). https://doi.org/10.1111/ele.13974

Fish and Wildlife Service (2010). "Mississippi Sandhill Crane National Wildlife Refuge: Unique Habitats." Retrieved October 12, 2021, from https://www.fws.gov/mississippisandhillcrane/habitats.html

FNAI (Florida Natural Areas Inventory). 2010. Guide to the natural communities of Florida: 2010 edition. Florida Natural Areas Inventory, Tallahassee, FL, US. https://doi.org/10.1016/j.foreco.2020.118539

Frost, C.C. 1998. Presettlement fire frequency regimes of the United States: a first approximation. Pages 70-81 in T.L. Pruden and L.A. Brennan, editors. Fire in Ecosystem Management: Shifting the Paradigm from Suppression to Prescription. Tall Timbers Fire Ecology Conference Proceedings, No. 20. Tall Timbers Research Station, Tallahassee, Florida.

Grace, J. (1998). Can prescribed fire save the endangered coastal prairie ecosystem from Chinese tallow invasion. *Endangered Species Update*, *15*.

Grace, James B.; Smith, Melinda D.; Grace, Susan L.; Collins, Scott L.; Stohlgren, Thomas J. 2001. Interactions between fire and invasive plants in temperate grasslands of North America. In: Galley, Krista E. M.; Wilson, Tyrone P., eds. Proceedings of the invasive species workshop: The role of fire in the control and spread of invasive species; Fire conference 2000: the first national congress on fire ecology, prevention, and management; 2000 November 27 - December 1; San Diego, CA. Misc. Publ. No. 11. Tallahassee, FL: Tall Timbers Research Station: 40-65.

Grace, James B.; Allain, Larry K.; Baldwin, Heather Q.; Billock, Arlene G.; Eddleman, William R.; Given, Aaron M.; Jeske, Clint W.; Moss, Rebecca. 2005. Effects of prescribed fire in the coastal prairies of Texas. USGS Open-File Report 2005-1287. Reston, VA: U.S. Department of the Interior, Fish and Wildlife Service, Region 2; U.S. Geological Survey. 46 p.

Gan, Jianbang; Miller, James H.; Wang, Hsiaohsuan; Taylor, John W., Jr. 2009. Invasion of tallow tree into southern US forests: influencing factors and implications for mitigation. Canadian Journal of Forest Research. 39: 1346-1356.

Grier C C, Vogt K A, Keyes M R and Edmonds R L 1981 Biomass distribution and above- and below-ground production in young and mature *Abies amabilis* zone ecosystem of the Washington Cascades. Can. J. For. Res. 11, 155–167. https://cdnsciencepub.com/doi/abs/10.1139/x81-021

Groeschl, D. A., Johnson, J. E., & Smith, D. W. (1993). Wildfire Effects on Forest Floor and Surface Soil in a Table Mountain Pine-Pitch Pine Forest. *International Journal of Wildland Fire*, *3*(3), 149–154. https://doi.org/10.1071/wf9930149

Hibdon, Lisa M. 2005. Litter dynamics and detrital food webs of native and Chinese tallow stands in a Louisiana bottomland hardwood forest. New Orleans, LA: University of New Orleans. 85 p.

Hoffmann, W. A., Adasme, R., Haridasan, M., T. de Carvalho, M., Geiger, E. L., Pereira, M. A. B., Gotsch, S. G., & Franco, A. C. (2009). Tree topkill, not mortality, governs the dynamics of savanna–forest boundaries under frequent fire in central Brazil. *Ecology*, *90*(5), 1326–1337. https://doi.org/10.1890/08-0741.1

Jubinsky, Greg. 1995. Chinese tallow (Sapium sebiferum). Report No. TSS-93-03. Tallahassee, FL: Florida Department of Environmental Protection, Bureau of Aquatic Plant Management. 12 p. [54504]

Keeley, J. E., & Keeley, J. E. (2009). Fire intensity, fire severity and burn severity: A brief review and suggested usage. *International Journal of Wildland Fire*, *18*(1), 116–126. https://doi.org/10.1071/WF07049

Keyser, T.L., and Zarnoch, S.J. 2014. Stump sprout dynamics in response to reductions in stand density for nine upland hardwood species in the southern Appalachian Mountains. Forest Ecology and Management 319: 29-35.

Kobe, R. K. (1997). Carbohydrate Allocation to Storage as a Basis of Interspecific Variation in Sapling Survivorship and Growth. *Oikos*, *80*(2), 226–233. https://doi.org/10.2307/3546590

Lin, W. C.; Chen, A. C.; Tseng, C. J.; Huang, S. G. 1958. An investigation and study of Chinese tallow tree in Taiwan (Sapium sebiferum Roxb). Bulletin of Taiwan Forestry Research Institute. 57: 32-37.

Lin, J., Harcombe, P. A., Fulton, M. R., & Hall, R. W. (2004). Sapling growth and survivorship as affected by light and flooding in a river floodplain forest of southeast Texas. *Oecologia*, *139*(3), 399–407. https://doi.org/10.1007/s00442-004-1522-6

Lloret, F., & López-Soria, L. (1993). Resprouting of Erica multiflora after Experimental Fire Treatments. *Journal of Vegetation Science*, *4*(3), 367–374. https://doi.org/10.2307/3235595

Matula, R., Šrámek, M., Kvasnica, J., Uherková, B., Slepička, J., Matoušková, M., Kutchartt, E., & Svátek, M. (2019). Pre-disturbance tree size, sprouting vigour and competition drive the survival and growth of resprouting trees. *Forest Ecology and Management*, *446*, 71–79. https://doi.org/10.1016/j.foreco.2019.05.012

Meyer, Rachelle. 2011. Triadica sebifera. In: Fire Effects Information System, [Online]. U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, Fire Sciences Laboratory (Producer). Available: https://www.fs.usda.gov/database/feis/plants/tree/triseb/all.html [2023, June 19].

Midgley, J. J., Kruger, L. M., & Skelton, R. (2011). How do fires kill plants? The hydraulic death hypothesis and Cape Proteaceae "fire-resisters." *South African Journal of Botany*, *77*(2), 381–386. https://doi.org/10.1016/j.sajb.2010.10.001

Miller, James H. 2003. Nonnative invasive plants of southern forests: A field guide for identification and control. Gen. Tech. Rep. SRS-62. Asheville, NC: U.S. Department of Agriculture, Forest Service, Southern Research Station. 93 p.

NOAA *NCEI U.S. Climate Normals Quick Access*. (n.d.). Retrieved October 4, 2021, from https://www.ncei.noaa.gov/access/us-climate-normals/#dataset=normals-annualseasonal&timeframe=30&location=MS&station=US1MSJC0012

NRCS *Web Soil Survey—Home*. (n.d.). Retrieved October 5, 2021, from https://websoilsurvey.sc.egov.usda.gov/App/HomePage.htm

Paudel, S., & Battaglia, L. L. (2015). The role of light, soil and human factors on the probability of occurrence of an invasive and three native plant species in coastal transitions of coastal Mississippi, USA. *Journal of Plant Ecology*, *8*(5), 491–500. https://doi.org/10.1093/jpe/rtu045

Pimentel, D., Zuniga, R., & Morrison, D. (2005). Update on the environmental and economic costs associated with alien-invasive species in the United States. *Ecological Economics*, *52*(3), 273–288. https://doi.org/10.1016/j.ecolecon.2004.10.002

Poyner, C. (2017). Overstory management to regenerate longleaf pine and coastal invasive Chinese tallow in fire ecosystems. *Thesis*.

R Core Team (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Renne, I. J., Spira, T. P., & Bridges, W. C. (2001). Effects of Habitat, Burial, Age and Passage through Birds on Germination and Establishment of Chinese Tallow Tree in Coastal South Carolina. *The Journal of the Torrey Botanical Society*, *128*(2), 109–119. https://doi.org/10.2307/3088733

Renne, I. J., Barrow, W. C., Randall, L. A. J., & Bridges, W. C. (2002). Generalized avian dispersal syndrome contributes to Chinese tallow tree (Sapium sebiferum, Euphorbiaceae) invasiveness. *Diversity and Distributions*, *8*(5), 285–295. https://doi.org/10.1046/j.1472-4642.2002.00150.x

Rockwood, D. L.; Geary, T. F. 1991. Growth of 19 exotic and two native tree species on organic soils in southern Florida. In: Center, Ted D.; Doren, Robert F.; Hofstetter, Ronald L.; Myers, Ronald L.; Whiteaker, Louis D., eds. Proceedings of the symposium on exotic pest plants; 1988 November 2-4; Miami, FL. Tech. Rep. NPS/NREVER/NRTR-91/06. Washington, DC: U.S. Department of the Interior, National Park Service: 283-302.

Scheld, Herbert W.; Cowles, Joe R. 1981. Woody biomass potential of the Chinese tallow tree. *Economic Botany*. 35(4): 391-397.

Siemann, E., & Rogers, W. (2006). Recruitment Limitation, Seedling Performance and Persistence of Exotic Tree Monocultures. *Biological Invasions*, *8*, 979–991. https://doi.org/10.1007/s10530-005-0825-9

Smith, M. G., Arndt, S. K., Miller, R. E., Kasel, S., & Bennett, L. T. (2018). Trees use more non-structural carbohydrate reserves during epicormic than basal resprouting. *Tree Physiology*, *38*(12), 1779–1791. https://doi.org/10.1093/treephys/tpy099

USDA-Natural Resource Conservation Service. (2011). Soil Quality Indicators: Soil Electric Conductivity. *PDF*. https://www.nrcs.usda.gov/sites/default/files/2022-10/Soil%20Electrical%20Conductivity.pdf

Vesk, P. A., & Westoby, M. (2004). Funding the Bud Bank: A Review of the Costs of Buds. *Oikos*, *106*(1), 200–208.

Vesk, P. A. (2006). Plant Size and Resprouting Ability: Trading Tolerance and Avoidance of Damage? *Journal of Ecology*, *94*(5), 1027–1034.

Von Fircks, Y., & Sennerby-Forsse, L. (1998). Seasonal fluctuations of starch in root and stem tissues of coppiced Salix viminalis plants grown under two nitrogen regimes. *Tree Physiology*, *18*(4), 243–249. https://doi.org/10.1093/treephys/18.4.243

Wang, H.-H., Grant, W. E., Swannack, T. M., Gan, J., Rogers, W. E., Koralewski, T. E., Miller, J. H., & Taylor, J. W. (2011). Predicted range expansion of Chinese tallow tree (*Triadica sebifera*) in forestlands of the southern United States: Range expansion of Chinese tallow tree. *Diversity and Distributions*, *17*(3), 552–565. https://doi.org/10.1111/j.1472-4642.2011.00760.x

Williams, K., Meads, M. V., & Sauerbrey, D. A. (1998). The roles of seedling salt tolerance and resprouting in forest zonation on the west coast of Florida, USA. *American Journal of Botany*, *85*(12), 1745–1752. https://doi.org/10.2307/2446509

Wiley, E., King, C. M., & Landhäusser, S. M. (2019). Identifying the relevant carbohydrate storage pools available for remobilization in aspen roots. *Tree Physiology*, *39*(7), 1109–1120. https://doi.org/10.1093/treephys/tpz051