

CHINESE TALLOW TREE (*TRIADICA SEBIFERA*) MANAGEMENT AND SEED  
BIOLOGY

By

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A THESIS PRESENTED TO THE GRADUATE SCHOOL  
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT  
OF THE REQUIREMENTS FOR THE DEGREE OF  
MASTER OF SCIENCE

UNIVERSITY OF FLORIDA

2016

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To my family

## ACKNOWLEDGMENTS

I would like to thank Andrea Christman, Dr. William Haller, Dr. Gregory MacDonald and Gary Kreitzer for inspiring me to pursue a career in invasive plant and weed science. Without their guidance and support none of this would have been possible.

I would like to thank Dr. Hector Perez and Dr. Kimberly Bohn for their continuous assistance during my master's research and Dr. Susan Stieve and Dr. Pablo Jourdan at The Ohio State University for providing their expertise in x-ray imaging of seeds. I also would like to thank Justin McKeithen, Michael Durham, Gary Kreitzer, Bob Querns, Rose and Hans VanHeuveln, and the 2014-2016 Weed Science graduate students for assisting me with field and lab work. Their assistance was crucial and I cannot thank them enough for the time that they shared with me.

Sites for conducting research are difficult to come by so thank you to Philip McMillan at Neal Land and Timber and Andrea Christman with the Florida State Parks Service for providing me with locations to perform my research.

I would also like to thank the Florida Fish and Wildlife Conservation Commission, and the UF/IFAS Center for Aquatic and Invasive Plants for providing me with the funding necessary to complete my research.

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Abstract of Thesis Presented to the Graduate School  
of the University of Florida in Partial Fulfillment of the  
Requirements for the Degree of Master of Science

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By

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August 2016

Chair: Gregory E. MacDonald  
Major: Agronomy

Chinese tallow tree (*Triadica sebifera*) is an invasive tree species present throughout the southeastern United States. Chinese tallow tree has proven to be difficult to manage due to its aggressive growth and prolific seed production. Chemical control is widely used to manage this species but current herbicide recommendations fail to effectively control Chinese tallow tree regrowth. Studies were conducted at 3 locations in Florida to evaluate several herbicides in combination with basal bark, hack and squirt and cut stump application methods. Herbicides tested included aminocyclopyrachlor, glyphosate, hexazinone, imazapyr and triclopyr ester. Aminocyclopyrachlor provided at least 90% control over all application methods while all other herbicides evaluated did not consistently control Chinese tallow tree.

Chinese tallow tree seedling emergence and seed bank longevity was monitored at 2 sites in Florida to provide a better understanding of long-term impacts. Seedling emergence occurred over a 2-3 month period during spring and varied regionally. A period of dormancy was observed between growing seasons as well as a significant

decline in seedling emergence in the second growing season indicating a possible seed bank longevity of 2-3 years.

Growth chamber germination and viability testing were performed on seeds collected at 2 week intervals, after capsule split, in Gainesville, FL in 2014 and 2015. Harvest date, the effect of 6 month storage and the timing of aril removal were evaluated. Harvest date and storage temperatures studied had no significant effect on seed germination. Germination of stored seeds declined, but aril removal resulted in a drastic increase in germination, increasing the longer the aril was present on the seed. There was also no significant difference in seed viability observed across all treatments. X-ray analysis of seeds inside the aril showed lack of viability to be directly related to aberrations in seed fill or compromised embryos. This latter effect attributed to 72% of the non-viable seeds. These seed studies indicate that dormancy is most likely present and influenced by the aril, but further research is needed to elucidate the mechanism of dormancy in Chinese tallow tree.

## CHAPTER 1 INTRODUCTION

Florida has over 4,000 plant species, of which 1,300 or more have been introduced to the state and are termed nonnative exotic species by the Florida Exotic Pest Plant Council (Anon. 2014 b). Of these non-native exotic species, 130 are considered to be invasive throughout Florida's natural areas. However, some consider the number of non-native invasive plant species to in Florida to be as high as 900 (Anon. 2014 b; McCormick 2005). Invasive species have been known to cause damage to natural communities and ecosystems through displacement of native species, modification of ecosystem structure, and function and decrease native species diversity which threatens extinction of some rare resident species (McCormick 2005). Such serious environmental impacts along with the potential for invasive plant species to threaten and impair public waters, natural and agricultural lands, has ultimately lead to considerable funding for invasive plant control in Florida. In 2005, \$80 million Florida tax dollars were spent on the control of all invasive plants in the Florida (Anon. 2014 b). In contrast, the economic loss predicted from just a single plant species, *Melaleuca quinquenervia*, over a 20 year period have been estimated as high as \$2 billion (McCormick 2005). Understanding how invasive species establish, spread and impact Florida's ecosystems is critical. Limiting and preventing spread, along with managing and restoring lands that have already been impacted will reduce the economic and ecological losses causes by these non-native invasive plant species.

Chinese tallow tree [*Triadica sebifera* (L.) Small, Euphorbiaceae, (synonyms include *Sapium sebiferum* (L.) Roxb and *Stillingia sebifera* Willd.)] is a sub-tropical, monoecious, deciduous tree native to Eastern Asia. First introduced to the United

States in the late 1770's and specifically to Charleston, South Carolina in the 1780's (Renne 2001), it has now naturalized and occurs from North Carolina south into Florida and west through Louisiana, Arkansas and Texas. Isolated populations have been reported in California (USDA 2016). This distribution is the result of its promotion to develop a commercial soap industry in coastal Louisiana and Texas by the Foreign Plant Introduction Division of the U.S. Department of Agriculture in the 1906, its rise in popularity as a colorful ornamental in the mid to late 1900's, and potential use as an oil seed and composite manufacturing crop (McCormick 2005).

In Florida, Chinese tallow tree has become a serious threat to many natural communities in the northern and central regions of the state. Which has led to its designation by the Florida Exotic Pest Plant Council in 1991 as a Category I exotic species, due to its ability to alter native plant communities and change community structures and ecological functions (McCormick 2005). In 1996, the Florida Department of Agricultural and Consumer Services listed it as a State Noxious Weed and prohibited its propagation, commerce and transportation in Florida in 1998 (McCormick 2005).

### **Biology**

Chinese tallow tree can establish in a wide range of habitats, but performs best in disturbed systems due to increased seed germination in environments with greater diurnal temperature fluctuations is caused by canopy gaps (Donahue 2004). It can establish in upland sites but is more likely to establish successfully in, open habitats; therefore causing problems in floodplain ecosystems (McCormick 2005). This woody invader grows very rapidly, with reported growth rates in canopy gaps of both coastal prairie ecosystems and Chinese tallow tree forests of 0.08 and 0.33 cm per day,

respectively (Butterfield et al. 2004). It has been observed to reach heights of 2.8 meters in 2 years (Cameron et al. 2000) and achieve heights of up to 12-13 meters in 12 to 13 years (McCormick 2005). Chinese tallow tree seedlings are able to grow at rates equal or greater than those of many native seedlings across a range of environmental conditions (Siemann & Rogers 2006). This species is able to tolerate shading (5% sunlight), moderate levels of salinity, flooding, and is relatively drought tolerant. The species range is apparently only limited by extremely arid regions, freezing temperatures and high salinity levels (Barrilleaux & Grace 2000).

While Chinese tallow tree can reproduce vegetatively through root sprouting, seeds are the main mechanism of spread and invasion. Trees have the ability to produce more than 100,000 seeds per tree (Anon. 2014 a) and produced 4,484kg ha<sup>-1</sup> yr<sup>-1</sup> in a Texas coastal prairie (Cameron et al. 2000). This high rate of seed production in combination with the high growth rates mentioned earlier allows Chinese tallow tree to quickly create mono-specific stands in as little as 20 years (Wang et al. 2001; Bruce et al. 1995).

Seeds develop within three-lobed, three-valve capsules which dehisce upon maturity, exposing the white and waxy seeds that can stay attached to the tree for extended periods of time (Breitenbeck 2009). Seeds are known to be dispersed by birds and water (Renne et al. 2001; Conway et al., 2002). Though much research has been reported regarding bird seed dispersal, few studies have explicitly examined the contribution of water dispersal. However, seed germination studies with conflicting results have reported on the effects of extended water soaking. Samuels (2004) found that water soaked seeds had significantly higher germination compared to seeds that

underwent an acid treatment to simulate bird digestion, while Bower (2009) found the opposite result.

Studies conducted to this date have not determined whether or not seeds require a period of dormancy. Seeds can remain viable for at least 7 years in cold storage (0-5 C), with field survival limited to 2 years (Renne et al. 2000; Cameron et al. 2000; Renne et al. 2001; Meyers 2011). Environmental conditions apparently also have an impact on germination. Seedlings appear to have peak emergence occurring in mid spring and then declining sharply in summer and fall. Seeds have highest germination under greenhouse conditions with large diurnal temperature fluctuations averaging  $13\pm1$  C under moist soils with or without light (Renne et al. 2001; Nijjer et al. 2002; Donahue, 2004; Bower 2009). The embryo itself does not appear to be dormant; Shu-xain et al. (2012) reported 80% seed germination when the embryo was excised from seed tissues. Other than environmental conditions, dormancy, if occurring, is thought to be regulated by the aril either through chemical inhibition or coat imposed dormancy (Candiani et al. 2004; Shu-xain et al. 2012). Removal of the aril increases germination (Shu-xain et al. 2012), but Siril et al. (1998) showed partial removal results in increased dormancy. This points out that the exact impact of how the aril influences seed germination is still not fully understood.

Overall, research on seed dormancy and germination in Chinese tallow tree has largely resulted from an ecological rather than a physiological perspective, and often falls short when trying to understand seed bank dynamics. Many studies have conducted seed germination and dormancy tests, but failed to account for population differences or did not complete the tests with enough variation of treatments (seed

research has typically not been the primary objective of the research). Studies which did use physiological approaches, reported inconclusive results caused by a lack of seed viability testing, small sample sizes, and incomplete population sampling (i.e. comparing seeds of trees at different stages of development) (Conway et al. 2000; Cameron et al. 2000). The mechanism of dormancy and methods to break dormancy is crucial to understanding how long seeds persist in the environment and will provide a greater understanding of the seed bank dynamics of this species which will hopefully led to more effective seedling management strategies for more effective control and site restoration.

### **Management**

Since Chinese tallow tree's designation as a Florida Noxious Weed in 1996, the state of Florida has spent over \$750,000 implementing management efforts to control Chinese tallow tree in north and central regions of the state. Other states with similar Chinese tallow tree infestations (Texas, Louisiana and Mississippi) are predicted to spend an additional \$200-400 million on Chinese tallow tree management in the next 20 years (Wheeler & Ding 2014). For this reason it is important to develop the most effective methods for controlling this species since a 5-10% improvement in control methodology can save millions of dollars in long term management programs.

An integrated management approach is typically used when managing invasive plant species. Integrated management approaches combine preventative, biological, cultural, mechanical and chemical methods to achieve a management objective (MacDonald et al. 2013; Jackson 2005; McCormick 2005). Currently, Chinese tallow tree management is not truly integrated since alternative methods have proven to be



ineffective or are still not fully developed. Thus, management of Chinese tallow tree presently relies primarily on chemical control (McCormick 2005).

Classical biological control uses introduced host-specific natural enemies to suppress the pest species (McCormick 2005; Tu et al. 2001). Such techniques have been highly successful controlling alligatorweed (*Alternanthera philoxeroides*) and tropical soda apple (*Solanum viarum*) but currently there are no biological control agents available for Chinese tallow tree (Van Driesche et al. 2002). There are several insect herbivores currently present and feeding on Chinese tallow tree in the United States, but only one known specialist feeding species. *Caloptilia tridicae* is a moth with leaf mining larvae which feed on Chinese tallow tree leaves (Wheeler & Ding 2014). Though it might be several years before a biological control agent is released, the prospects are promising. Chinese tallow tree is a phylogenetically isolated species in the United States, thus reducing the risk for damage to native species by any introduced biological control agents. There are also many known Chinese tallow tree pathogens and associated insect species in China which may provide good candidates for biocontrol of this species (Wheeler & Ding 2014).

Prescribed fires and hydrological manipulation are cultural methods of control that have yet to provide effective control for Chinese tallow tree. Prescribed fires only control seedlings and saplings, and provide only in top kill for adult specimens. Fire also has the potential to make burned locations more vulnerable to reinvasion through re-sprouting and improved site conditions for seed germination (Grace 1998, McCormick 2005, Miller et al. 2015). Hydrologic manipulation has not been extensively studied due to its difficulty to implement on a large scale (McCormick 2005), but

observational evidence suggests flooding might be effective (G. E. MacDonald, pers. comm). Mechanical control of Chinese tallow tree is also ineffective since root fragments can produce new sprouts and cut surfaces can coppice extensively if not chemically treated. These activities also usually heavily disturb soils, increasing susceptibility to reinvasion and erosion (Urbatsch 2000; McCormick 2005; Miller et al. 2015). Chemical control at this time offers the greatest and most cost effective control by effectively targeting below and above ground plant biomass and causing the least amount of soil disturbance (Miller et al. 2015).

Chinese tallow tree in natural areas, can form dense mono-typic stands but more commonly is found in mixed stands which limits over-the-top broadcast herbicide applications due to use of non-selective herbicides resulting in damage to desirable species. Therefore basal bark, hack and squirt and cut stump application methods are likely to be the most selective and therefore are commonly used for herbicide treatments in natural areas. Current herbicide recommendations fail to include newly registered herbicides and lack supporting peer reviewed literature that addresses treatment effectiveness initially and how well re-sprouting is controlled. Enloe et al. (2015) has reported that triclopyr, a selective systemic herbicide used commonly for Chinese tallow tree management has unreliable control and was out performed by several newly registered herbicides. The need to evaluate additional herbicides and revisit application techniques with commonly used herbicides such as glyphosate, hexazinone and imazapyr is highly warranted to determine the most effective herbicide, rate and application method to manage Chinese tallow tree.

## **Research Objectives**

These experiments are designed to provide a more comprehensive understanding of Chinese tallow tree seed biology and management in central and north Florida ecosystems. Specific goals include: 1) determining seedling emergence patterns and seed bank longevity to define post treatment site monitoring recommendations; 2) evaluating seed germination and viability as a factor of duration seed was present on and off tree and timing of aril removal after capsule split to further understand seed germination and the potential for seed dormancy; and 3) comparing basal, hack and squirt and cut stump application methods with herbicide to address knowledge gaps in treatment strategies.

## CHAPTER 2

### EVALUATION OF APPLICATION METHOD AND COMMON HERBICIDES FOR MANAGEMENT OF CHINESE TALLOW TREE (*TRIADICA SEBIFERA*)

#### **Background**

Chinese tallow tree [*Triadica sebifera* (L.) Small, Euphorbiaceae, (synonyms include *Sapium sebiferum* (L.) Roxb and *Stillingia sebifera* Willd.)] is a sub-tropical, monoecious, deciduous tree native to Southeastern Asia (McCormick 2005). First introduced to the United States in the late 1770's, Chinese tallow tree has since become an aggressive invasive species which is known to invade a wide range of intact and disturbed ecosystems throughout the Southeastern United States (McCormick 2005). Its current range extends from North Carolina, south into Florida and west through Louisiana, Arkansas and Texas with isolated populations occurring in Southern California (USDA 2016). This region of the US is known to have some of the most productive forestry sites in the country. In 2005, the southeast alone maintained over 87 million ha of forests which produce over 60% of the timber grown in the US (Gan et al. 2009). In 2008, there were over 185,000 ha of southern US forests reportedly invaded with Chinese tallow tree (Gan et al. 2009) and with its range predicted to expand to 1.58 million ha by 2023 (Wang et al. 2011). As Chinese tallow tree's environmental range expands and infestations become more extreme, the economic cost for managing Chinese tallow tree is also predicted to increase. From 1998-2007 Florida spent nearly \$1 million managing 2,023 ha of natural areas in the Northern and Central regions of the state which is approximately 1% of the acreage impacted in southern forests in 2008 (Wheeler & Ding 2014). Within the next 20 years Texas, Louisiana and Mississippi are predicted to spend \$200-400 million managing lands infested with Chinese tallow tree (Wheeler & Ding 2014). Therefore, it is imperative to

develop the most effective means of control for this species and improve upon existing technologies to make treatments successful as possible to reduce the overall cost of management.

Chinese tallow tree has proved difficult to manage due to production of copious seeds, root and stump re-sprouting or coppicing following herbicide treatments. The most effective control of Chinese tallow tree is achieved through the use of herbicides (McCormick 2005). Physical and cultural control techniques such as prescribed fire and mechanical removal of Chinese tallow tree typically result in only temporary control, allowing the tree to aggressively re-sprout from living underground roots or the stump (Urbatsch 2000; McCormick 2005; Miller et al. 2015; Del Tredici 2001). Complete kill is necessary to reduce or eliminate regrowth that results in a multi trunked tree that requires increased effort and chemical to treat. For this reason an integrated approach for controlling Chinese tallow tree and other similar invasive plants is desirable but currently not available. Thus, additional research is needed to identify the most effective chemical treatments to provide more effective control of this species (MacDonald et al. 2013; Jackson 2005).

Site complexity in natural areas governs methods used to maintain selectivity and provide control of large specimens with minimal off-target damage to non-target species (MacDonald et al. 2013). These management goals can be achieved by choosing the herbicide which has the least impact on desirable species and with the most effective application methods such as basal bark, hack and squirt and cut stump treatments (MacDonald et al. 2013).

Basal bark treatments, which use an herbicide and penetrant oil mixture to carry chemical through the bark of woody plants, are recommended to be used on woody plants with stem/trunk diameters <20 cm but may be used on larger trees if highly susceptible (MacDonald et al. 2013; Miller et al. 2015). This method applies a 6-12 inch band of herbicide around the circumference of the trunk, with the width of the spray band depending on the susceptibility and size of the target species (Tu et al. 2001). Herbicides that are effective with basal bark applications are those which are oil soluble and travel systemically throughout the plant (MacDonald et al. 2013). Basal bark treatments in the south-eastern US are often applied in June-September while trees are actively growing (Miller et al. 2015).

Hack and squirt treatments, a crude form of stem injection, is considered one of the most cost effective means of controlling many invasive tree species (Miller et al. 2015). This technique is commonly used on >5 cm diameter trees and is useful where complete basal applications might be difficult (Tu et al. 2001; Miller et al. 2015). This method uses a cutting tool and a small amount of undiluted chemical which is directly applied to the living tissue (Tu et al. 2001). Cuts are made into the outer bark at a 45 degree angle, penetrating into cambium layer, creating a cup/flange where the herbicide is then placed (MacDonald et al. 2013). This cup-like fringe of peeled bark holds the herbicide in contact with the cambial layer (phloem), increasing exposure time and allowing direct up take by the plant. Hack and squirt treatments can be applied year round but are least effective during periods of drought or prolonged freezing conditions (Miller et al. 2015).

Cut stump treatments can be used on small or large diameter trees but are typically used when the plant causes a nuisance such as being an unsightly element to a landscape or a potential safety hazard. Cut stump treatments require felling the tree and applying undiluted concentrated herbicides or herbicide oil/water mixtures to the outer cambium layer of the stump (Tu et al. 2001; MacDonald et al. 2013). Herbicide should be applied to a clean, flat surfaced stump within 5-30 minutes of cutting to insure adequate uptake (Tu et al. 2001). Cut stump treatments can be employed at any time throughout the year but summer and fall applications typically provide the most effective control (Miller et al. 2015).

Post emergent broadleaf herbicides that are labelled and commonly selected for control of broadleaf invasive woody shrubs and trees include imazapyr, triclopyr, glyphosate and hexazinone. Aminopyralid, fluroxypyr, imazamox and aminocyclopyrachlor are several newer herbicides that continue to be evaluated in recent years for natural area weed control on a range of invasive species (MacDonald et al. 2013; Enloe et al. 2015). This renewed interest in research/evaluation of alternative herbicides for invasive plant management is due in part to the establishment of the National Invasive Species Council and the Invasive Species Advisory Committee in 1999 which mandated that invasive species and their impacts be addressed (MacDonald et al. 2013).

Currently, herbicide recommendations for management of Chinese tallow tree and many other natural area weeds have been developed from anecdotal field observations and based largely on outdated herbicide recommendations (Enloe et al. 2015). Typically, triclopyr ester formulations are recommended for basal bark or foliar

treatment, triclopyr amine formulations for cut stump and foliar treatments and imazapyr for foliar and hack and squirt treatments (Enloe et al. 2015). However, these current recommendations often require follow-up applications to control re-sprouting that commonly occurs (Enloe et al. 2015; Urbatsch 2000). Recent research by Enloe et al. (2015) and Yiser et al. (2012) showed that triclopyr, one of the most highly recommended herbicides for Chinese tallow tree control, provides inconsistent results over a range of application techniques. Enloe et al. (2015) evaluated several recently registered herbicides for natural areas and showed promising results for aminocyclopyrachlor for Chinese tallow tree which was also reported by Yiser et al. (2012).

The research objectives of this study is to expanded the scope of Enloe et al. (2015) studies to determine the most effective herbicide treatments as a function of application method; thus providing additional quantitative data to develop more effective treatment options for this species.

## **Materials and Methods**

### **Site Descriptions**

Herbicide studies to evaluate the effectiveness of basal bark, hack and squirt and cut stump for the control of Chinese tallow tree were established at 3 locations in northwest and central Florida. The first site was located near the West Florida Research Education Center (WFREC) in Jay, Florida (30°45'52.31"N, 87°02'27.60"W) and mainly consisted of a cypress hammock adjacent to fields of row crops. This site typically has standing water. Tree species in the cypress hammock include Chinese tallow tree, sweetgum (*Liquidambar styraciflua*) and cypress (*Taxodium distichum*). The second site, located at Paynes Prairie Preserve Florida State Park (PPP) in Gainesville, Florida



(29°36'49.16"N, 82°20'04.19"W), is a wet prairie surrounded by a basin marsh. Due to land use/monitoring changes the wet prairie itself has been overrun with mixed hardwoods with traces of species which probably once dominated the area. These include pickerelweed (*Pontederia cordata*), maidencane (*Panicum hemitomon*) and other grass and sedge species, but now is primarily dominated by Chinese tallow tree and southern blackberry (*Rubus argutus*) with a few scattered, sweetgum (*Liquidambar styraciflua*), red maple (*Acer rubrum*) and persimmon (*Diospyros virginiana*) trees. The research site did have some small areas where standing water occurred but was primarily dry throughout the duration of these studies (2013-2015). The final site was located at Neal Land and Timber (NLT) in Blountstown, Florida (30°25'36.42"N, 85°00'12.40"W). This flood plain consisted of bottom land hardwood swamp with portions developed into a pine plantation. The site consisted of magnolia, gum, bay, maple and oak species with Chinese tallow tree and Japanese climbing fern (*Lygodium japonicum*) invading the site.

### **Experimental Design**

All studies were initiated in December 2013 through January 2014 and repeated in December 2014/January 2015. Studies were conducted using a completely randomized experimental design (CRD) with each individual Chinese tallow tree assigned as a single experimental unit/replication. Each treatment was replicated 6 times, with a minimum of 1 meter spacing between treated trees. Application methods were assigned based on the trunk circumference and diameter at breast height (DBH) of each tree which was measured and recorded prior to application. After categorizing trees by size, treatments were randomly assigned to each tree.

Cut stump and hack and squirt herbicide treatments included undiluted aminocyclopyrachlor (Method® 240SL, DuPont, Wilmington, DE 19898), imazapyr (Chopper® Gen2, BASF, Research Triangle Park, NC 27709), 1:1 mix of aminocyclopyrachlor and imazapyr, glyphosate (Accord®, Dow Agro Sciences, Indianapolis, IN 46268), hexazinone (Velpar L®, DuPont Wilmington, DE 19898) and triclopyr ester (Garlon® 4 Ultra, Dow AgroSciences, Indianapolis, IN 46268). Basal bark included all herbicides listed above with the exception of hexazinone and glyphosate, due to the lack of oil soluble formulations (MacDonald et al. 2013). General descriptions of each herbicide are found in Table 2-1 and rates for each herbicide treatment are listed in Tables 2-3, 2-4, 2-5 for each treatment method.

### **Basal Bark**

Basal bark treatments were applied to trees with a DBH of less than 10 cm. Herbicides were combined with JLB Oil Plus® penetrant oil (Brewer International, Inc. Vero Beach, FL 32968 ) and applied in a complete 15-20 cm wide band around the base (approximately 30 cm from the soil surface) of each tree trunk using a hand sprayer.

### **Hack and Squirt**

Hack and squirt treatments were applied using a small hatchet to “hack” 5 cm wide 45 degree flanges into the side of each tree into which 1 ml of concentrated herbicide was applied with a syringe. Cuts were made deep enough to penetrate through the bark and into the living cambium (Jackson 2005), which allowed herbicide uptake into the plant via passive diffusion into the xylem and phloem. The number of hacks each tree received was determined by circumference. Trees having a

circumference of 15-23 cm, 24-56 cm and 57-87 cm received 1, 2 and 3 hacks respectively, which were equally spaced around each trunk and not overlapping per University of Florida extension recommendations (Ferrell et al. 2006).

### **Cut Stump**

Cut stump treatments were applied to individual trees with a DBH of at least 16 cm. Trees were felled using a chainsaw, leaving a flat surfaced stump that was approximately 15 cm above ground level. The stump was wiped clean and herbicide was applied within 5 minutes of the felling of each tree. The herbicide was applied in a band 2X around the cambium layer applying approximately 8-10 ml of herbicide to each stump.

### **Analysis**

Visual control ratings of the canopy of each tree were used to quantify effective control using a 0-100% scale. Trees rated as 0% showed no visual sign of damage when compared to untreated site trees. A 50% rating indicated a 50% reduction in canopy cover and visual signs of herbicide damage such as chlorosis, abnormal growth and necrosis; and 100% represented tree death with a lack of root sprouting within 0.5 m around the treated tree base. To confirm tree death, bark was removed down to the inner bark, if green living tissue was observed the tree was not rated as 100%. Ratings were taken 6 and 10 months after treatment (MAT). Data was analyzed in S.A.S. 9.4 using the PROC GLM statement to provide analysis of variance and means. Treatment means were separated using Fisher's Protected Least Significant Difference (LSD) test with a 0.05 probability. There were no significant difference between years ( $P > 0.05$ ) so years were pooled within treatments for each location (Table 2-2).

## Results and Discussion

### Basal Bark

Basal bark treatments were significantly different among herbicide treatments and between locations at 6 MAT and 10 MAT ( $P \leq 0.001$ ), therefore data is presented separately for each location (Table 2-3). All treatments provided reduced foliar cover or complete control with no evidence of root collar regrowth. Aminocyclopyrachlor alone or in combination with imazapyr provided the most consistent control when applied as a basal bark treatment. Control with aminocyclopyrachlor alone 6 MAT ranged from 84-100% across the three locations, with similar results 10 MAT (86-100%) (Table 2-3). The combination of imazapyr and aminocyclopyrachlor did not result in increased control compared to aminocyclopyrachlor alone. Triclopyr provided control comparable to aminocyclopyrachlor at the NLT and PPP locations, but worked as effectively as imazapyr only at the WFREC location. Imazapyr provided the least amount of control compared to the other herbicide treatments, with <60% control at the NLT and PPP sites 10 MAT.

Overall, the WFREC site showed higher levels of control than treatments at the other 2 locations. This is possibly due to the size of trees selected. Trees at the WFREC site had a mean trunk circumference of 3.38 cm  $\pm$  SD 0.79 while PPP and NLT had mean trunk circumferences of 15.42 cm  $\pm$  SD 3.78 and 12.6 cm  $\pm$  SD 7.24 respectively. Smaller trees can be controlled with less chemical thus it is likely that this played a major role in the amount of control seen at the WFREC site (Miller et al. 2015).

Flooding also could have contributed to the differences in control seen at the WFREC site. Between March and April of 2014 and 2015 the WFREC site experienced higher mean rainfall than all other sites (Figure 2-1). Though no site data was collected

in terms of water depth or duration standing water was present; standing water was observed over an extended period of time at this site (G. E. MacDonald, pers. comm.). Flooding for long periods of time can decrease soil O<sub>2</sub> which is necessary for root respiration and can lead to reduction in growth and typically tree death (Pallardy 2006). This in combination with herbicide treatments, probably led to the near complete control of all tree replicates. Therefore, the Neal Land and Timber and Paynes Prairie locations most likely represent a more accurate depiction of how these herbicides will perform, without the complications of flooding stress. This also suggests a physical stress (flooding) should be studied as a potential management tool to improve herbicide effectiveness where flooding is possible.

### **Hack and Squirt**

Hack and squirt treatments evaluated 6 MAT were significantly different for herbicide treatment ( $P > .001$ ), but not location ( $P = 0.6702$ ) with no significant treatment location interaction ( $P < 0.0675$ ) (Table 2-4). Aminocyclopyrachlor, imazapyr and the combination provided the highest level of control for hack and squirt treatments ( $\geq 97\%$ ) across all sites (Table 2-4). Glyphosate provided control similar to aminocyclopyrachlor treatments at WFREC and PPP, but performed comparable to triclopyr at NLT. Overall control from glyphosate ranged from 81-98%. Triclopyr provided inconstant results when applied using the hack and squirt method with control means ranging from 67-92% across the 3 locations. Hexazinone treatments provided the least amount of control with mean visual ratings of 50-56% at 6 MAT.

At 10 MAT, treatment ( $P < 0.0001$ ), location ( $P = 0.0024$ ) and their interaction ( $P = 0.0248$ ) were all significantly different (Table 2-4). Treatments at WFREC were more effective than treatments at the other 2 sites which is possibly is due to using

smaller trees at this site. Mean circumferences of tree trunks at NLT and PPP sites where  $20.5 \text{ cm} \pm \text{SD } 11.12$  and  $29.9 \text{ cm} \pm \text{SD } 8.3$  respectively compared to the trees at WFREC ( $5.6 \text{ cm} \pm \text{SD } 1.4$ ). As mentioned previously smaller trees might respond more quickly and effectively to herbicide treatments but the number of hacks which was determined by circumference should have minimized this effect (Miller et al. 2015). Treatments containing aminocyclopyrachlor and/or imazapyr consistently provided statistically similar control, with 100% control at the WFREC and PPP locations and  $\geq 96\%$  observed at NLT. Control from imazapyr provided control just as effective as both aminocyclopyrachlor alone and aminocyclopyrachlor combined with imazapyr. Glyphosate treatments showed variable control across locations ranging from 92% at WFREC to 71% and 77% at NLT and PPP respectively performing as good as all other herbicides except triclopyr and hexazinone at WFREC, as good as triclopyr and better than hexazinone at NLT and performing as good as all other herbicides except hexazinone at PPP. Control from triclopyr was also variable, with the lowest level of control (61%) at the PPP site and 86% at NLT. This level of variability is consistent with reports from land managers (Enloe et al. 2015).

Hexazinone 10MAT showed approximately 50% control at the NLT and PPP sites, but at the WFREC control was comparable to most other treatments at 86%. Hexazinone is a soil active chemical that is water soluble and readily absorbed by plant roots and moved through the xylem apoplastically (WSSA 2014). Since it only moves in the xylem, high levels of herbicide exposure are not likely in tissues that are below each hack since solutions within the xylem only flows acropetally. It is possible that since there was a high incidence of rainfall at WFREC, as mentioned in the basal bark study,

it is very likely that enough hexazinone was washed out of the hacks and into the soil, which potentially allowed the roots and the area below the hacks to be exposed to the herbicide leading more effective control (Tu et al. 2001).

Therefore, both aminocyclopyrachlor and imazapyr treatments provide adequate results to use either inter-changeably or in combination when treating Chinese tallow tree using the hack and squirt application method. These herbicides provide satisfactory control of Chinese tallow tree, leading to complete tree death nearly 100% of the time. Results of both glyphosate and triclopyr (62-94% control 10 MAT) carried widely 10 MAT and will likely require multiple treatments for long term control. Hexazinone provided even less control in 5 of the 6 evaluations of the hack and squirt method.

### **Cut Stump**

Cut stump treatments were significantly different among and between treatment and location 6 MAT (Table 2-5). At 6 MAT triclopyr, imazapyr and both aminocyclopyrachlor treatments provided 93-100% control at all 3 sites. 10 MAT there was no significant difference in control between all 3 sites and these 4 treatments still provided excellent control ranging from total tree death to 88% control (Table 2-5). Glyphosate had variable results observed 6 MAT across locations while 10 MAT it consistently performed worse than the 4 more effective herbicide treatments and performed similar to or better than hexazinone. Hexazinone provided poor control at WFREC and PPP, but similar control to glyphosate at NLT.

At 10 MAT only herbicide treatments were found to be significantly different (Table 2-5). Triclopyr, imazapyr and both aminocyclopyrachlor treatments provided good to excellent control (>90%) with control from glyphosate and hexazinone ranging

from 61-71% to 43-47% across the locations, respectively. At 10 MAT the majority of hexazinone treatments developed coppiced stumps with only 16% of treated trees being 100% controlled. As mentioned in the hack and squirt study, a possible explanation for the poor performance of hexazinone is that it is an apoplastic, water soluble herbicide that is usually absorbed through the roots and translocated systemically through the xylem (WSSA 2014). Since the flow of water, and in this case hexazinone, through the xylem is only carried up, and not down towards the roots, this is probably the reason why these treatments were so ineffective (Pallardy 2006). If hexazinone was applied to the soil surface we might have seen better control, but this also would have increased the possibility of off target damage to surrounding plants (Tu et al. 2001).

These results collectively illustrate the effectiveness of aminocyclopyrachlor on Chinese tallow tree across all application methods. Imazapyr and triclopyr provided less consistent control across locations and application methods. Glyphosate and hexazinone were only marginally effective on Chinese tallow tree at our locations as a cut-stump and hack and squirt application and most likely will require retreatment.

These results suggest there is a need to continue to evaluate current and newly developed herbicides for Chinese tallow tree and other invasive plant species to generate quantitative data on effectiveness. This will then lead to improved herbicide recommendations for land managers to maximize the effectiveness of herbicide treatments.



Table 2-1. Descriptions of herbicides used for broadleaf tree control.

Trade name	Common name	Chemical family	Mode of action
Chopper®	Imazapyr	Imidazolinone	ALS/AHAS inhibitor
Garlon 4®	Triclopyr	Pyridine	Auxin Growth Regulator
Accord®	Glyphosate	Triazine	EPSPS Inhibitor
Velpar L®	Hexazinone	Triazine	PSII Inhibitor
Method®	Aminocyclopyrachlor	Pyrimidine carboxylic acid	Auxin Mimic Growth Regulator

Table 2-2. Analysis of variance for Chinese tallow tree (*Triadica sebifera*) control 6 and 10 months after treatment (MAT) for herbicide trials utilizing 3 application methods pooled across location and year P-values for type 1 error.

Source	Basal Bark			Hack and Squirt			Cut Stump		
	DF	6 MAT	10 MAT	DF	6 MAT	10 MAT	DF	6 MAT	10 MAT
Model	16	<0.0001	<0.0001	22	<0.0001	<0.0001	22	<0.0001	<0.0001
R-Sq		0.327	0.376		0.5	0.3618		0.673	0.53
Rep	5	0.8108	0.8525	5	0.8894	0.8876	5	0.53	0.8875
Treatment	3	0.0017	<0.0001	5	<0.0001	<0.0001	5	<0.0001	<0.0001
Location	2	<0.0001	<0.0001	2	0.6702	0.0024	2	<0.0001	0.2849
Interaction	6	0.131	0.0039	10	0.0675	0.0248	10	<0.0001	0.9849

Table 2-3. Mean visual ratings (0%=No effect, 100%=Tree death) of herbicides on Chinese tallow tree (*Triadica sebifera*) following basal bark treatments with diluted herbicide at 3 locations in Florida 6 and 10 months after treatment (MAT). WFREC= West Florida Research and Education Center, Jay, FL; NLT=Neal Land and Timber, Blountstown, FL; PPP=Paynes Prairie Preserve Florida State Park, Gainesville, FL.

Herbicide	Rate <sup>1</sup> % v/v	WFREC		NLT		PPP	
		6MAT	10MAT	6MAT	10MAT	6MAT	10MAT
		Control %					
Aminocyclopyrachlor	5	100 <sup>a2</sup>	100 <sup>a</sup>	98 <sup>a</sup>	93 <sup>a</sup>	84 <sup>a</sup>	86 <sup>a</sup>
Aminocyclopyrachlor + Imazapyr	2.5+0.5	100 <sup>a</sup>	99 <sup>a</sup>	98 <sup>a</sup>	87 <sup>a</sup>	81 <sup>a</sup>	85 <sup>a</sup>
Imazapyr	10	100 <sup>a</sup>	98 <sup>ab</sup>	76 <sup>b</sup>	43 <sup>b</sup>	55 <sup>b</sup>	57 <sup>b</sup>
Triclopyr	20	98 <sup>a</sup>	89 <sup>b</sup>	93 <sup>a</sup>	84 <sup>a</sup>	85 <sup>a</sup>	83 <sup>a</sup>
LSD <sub>0.05</sub>		2.4	9.5	14.4	18.7	25.7	24.9

<sup>1</sup> Treatments applied with JLB Oil Plus® Brewer International, Inc. Vero Beach, FL.

<sup>2</sup> Values reflect the mean of 6 replications. Means within a column followed by different letters are significantly different at P<0.05 according to Fisher's Least Significant Difference (LSD) test.

Table 2-4. Mean visual ratings (0%=No effect, 100%=Tree death) of undiluted herbicides on Chinese tallow tree (*Triadica sebifera*) following hack-and-squirt treatments with herbicide concentrate at 3 locations in Florida 6 and 10 months after treatment (MAT). WFREC= West Florida Research and Education Center, Jay, FL; NLT=Neal Land and Timber, Blountstown, FL; PPP=Paynes Prairie Preserve Florida State Park, Gainesville, FL.

		WFREC		NLT		PPP	
Herbicide	Rate	6MAT	10MAT	6MAT	10MAT	6MAT	10MAT
	ml undiluted	Control %					
Aminocyclopyrachlor	0.5	100 <sup>a1</sup>	100 <sup>a</sup>	100 <sup>a</sup>	100 <sup>a</sup>	100 <sup>a</sup>	100 <sup>a</sup>
Aminocyclopyrachlor + Imazapyr	0.5+0.5	100 <sup>a</sup>	100 <sup>a</sup>	98 <sup>a</sup>	96 <sup>a</sup>	100 <sup>a</sup>	100 <sup>a</sup>
Imazapyr	1.0	100 <sup>a</sup>	100 <sup>a</sup>	100 <sup>a</sup>	87 <sup>a</sup>	97 <sup>a</sup>	88 <sup>a</sup>
Triclopyr	1.0	71 <sup>b</sup>	74 <sup>b</sup>	92 <sup>ab</sup>	86 <sup>ab</sup>	67 <sup>b</sup>	61 <sup>bc</sup>
Glyphosate	1.0	98 <sup>a</sup>	92 <sup>a</sup>	81 <sup>b</sup>	71 <sup>bc</sup>	90 <sup>a</sup>	77 <sup>ab</sup>
Hexazinone	1.0	50 <sup>c</sup>	86 <sup>ab</sup>	56 <sup>c</sup>	56 <sup>c</sup>	56 <sup>b</sup>	48 <sup>c</sup>
LSD <sub>0.05</sub>		13.4	14.8	15.1	15.5	17.7	24.8

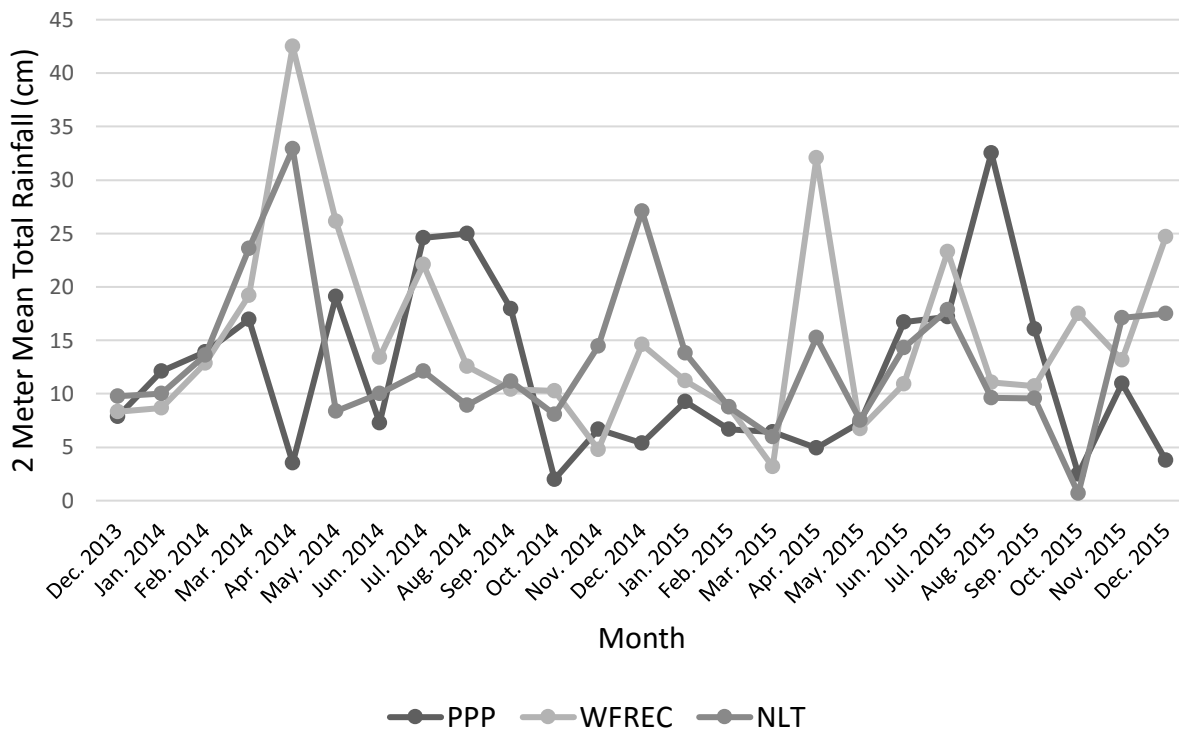
<sup>1</sup> Values reflect the mean of 6 replications. Means within a column followed by different letters are significantly different at P< 0.05 according to Fisher's Least Significant Difference (LSD) test.

Table 2-5. Mean visual ratings (0%=No effect, 100%=Tree death) of undiluted herbicides on Chinese tallow tree (*Triadica sebifera*) following cut stump treatments with herbicide concentrate at 3 locations in Florida 6 and 10 months after treatment (MAT). WFREC= West Florida Research and Education Center, Jay, FL; NLT=Neal Land and Timber, Blountstown, FL; PPP=Paynes Prairie Preserve Florida State Park, Gainesville, FL.

Herbicide	WFREC		NLT		PPP	
	6MAT	10MAT	6MAT	10MAT	6MAT	10MAT
	Control %					
Aminocyclopyrachlor	100 <sup>a1</sup>	100 <sup>a</sup>	100 <sup>a</sup>	100 <sup>a</sup>	100 <sup>a</sup>	100 <sup>a</sup>
Aminocyclopyrachlor + Imazapyr	100 <sup>a</sup>	100 <sup>a</sup>	100 <sup>a</sup>	100 <sup>a</sup>	100 <sup>a</sup>	99 <sup>a</sup>
Imazapyr	100 <sup>a</sup>	100 <sup>a</sup>	100 <sup>a</sup>	100 <sup>a</sup>	93 <sup>a</sup>	88 <sup>a</sup>
Triclopyr	99 <sup>a</sup>	100 <sup>a</sup>	100 <sup>a</sup>	94 <sup>a</sup>	97 <sup>a</sup>	91 <sup>a</sup>
Glyphosate	99 <sup>a</sup>	71 <sup>b</sup>	87 <sup>b</sup>	68 <sup>b</sup>	55 <sup>b</sup>	61 <sup>b</sup>
Hexazinone	47 <sup>b</sup>	43 <sup>c</sup>	84 <sup>b</sup>	47 <sup>c</sup>	27 <sup>c</sup>	43 <sup>b</sup>
LSD <sub>0.05</sub>	10.2	16.6	9.6	15.6	17.2	20

<sup>1</sup> Values reflect the mean of 6 replications. Means within a column followed by different letters are significantly different at P< 0.05 according to Fisher's Least Significant Difference (LSD) test

Figure 2-1. Florida Automated Weather Network (FAWN) 2m mean total rainfall data for 3 locations in Florida corresponding to locations near Chinese tallow tree (*Triadica sebifera*) research locations. PPP= Paynes Prairie Preserve Florida State Park, Gainesville, FL readings taken from Alachua Florida FAWN Station; NLT= Neal Land and Timber, Blountstown, FL with readings taken from the Quincy, FL FAWN Station; WFREC= West Florida Research and Education Center, Jay, FL with readings taken from the Jay, FL FAWN Station.



## CHAPTER 3 SEED BIOLOGY OF CHINESE TALLOW TREE (*TRIADICA SEBIFERA*)

### **Background**

In the United States, Chinese tallow tree has naturalized throughout much of the southeastern region of the country, spreading as far north as Arkansas and North Carolina and reaching south into southern Texas and South Florida (Urbatsch 2000; USDA 2016; Wunderlin & Hansen 2003). This broadleaf subtropical tree, native to Southeast Asia, grows approximately 1m per year, produces seeds within 3-8 years and can remain reproductive for approximately 100 years (Meyer 2011). Chinese tallow tree can develop monotypic tree stands within 18-30 years and gradually displaces native flora and fauna; eventually altering established ecosystem processes (Meyer 2011; Urbatsch 2000; Bruce et al. 1995).

This tree is capable of surviving in a wide range of environments but is commonly found in wet low lying areas, along waterbodies and dry uplands, with range limited by extended periods of extreme cold and/or arid conditions (Urbatsch 2000; Gan et al. 2009; Bruce 1993). Chinese tallow tree has also become invasive in its native range and is considered a weedy species in Taiwan (Langeland & Burks 1998). It has been considered an invasive species in the Carolinas since the 1970's and is a state noxious weed in Florida, Louisiana, Mississippi and Texas and currently recognized as the highest ranking category of invasive threat by all but one of the state chapters of the Southeast Exotic Plant Pest Council (SE-EPPC) (Langeland & Burks 1998; Langeland 2009; Anon. 2016).

Chinese tallow tree invasion success and persistence in the southeastern United States can be attributed to prolific seed production. The seeds of Chinese tallow tree

are held within a capsular fruit which contains 3 hard coated seeds and each seed is fully covered with a white tallow aril (Urbatsch 2000). The lipid rich aril is known to aid in hydrochory (dispersion of seeds via water movement) through increasing buoyancy, as well as encouraging zoochory by birds (Conway et al. 2002). Mature trees have been shown to produce on average 100,000 seeds per year and up to 300,000 or more seeds per year under ideal conditions (Bruce et al. 1997). Previous research indicates seed viability initially ranges from 88-95% with seed germination typically greater than many native tree species found in the southeastern United States. Seed germination has also been shown to occur across a much narrower range of conditions (Renne 2001; Cameron et al. 2000; Renne et al. 2001; Meyer 2011). Tests have shown Chinese tallow tree seeds can maintain viability in the field for at least 2 years in coastal South Carolina habitats and when placed in cold (0 C - 4 C) dry storage 12% of seeds germinated after 7 years (Renne 2001; Cameron et al. 2000; Renne et al. 2001; Meyers 2011). This suggests seeds have the ability to survive over multiple seasons and establish a soil seed bank but the potential seed bank longevity and the mechanisms controlling seed survival are still not understood (Renne et al. 2001).

Seed viability and dormancy could be influenced by the waxy seed aril. This seed structure is known to have the capacity to strongly influence germination in other species, acting as a physical barrier and/or trigger to germination or placing the seed in a state of dormancy (Candiani et al. 2004; Atwell et al. 1999). However, little is known concerning the impact of the aril on seed germination or survivability of Chinese tallow tree seeds. Previous research has suggested that seed germination of Chinese tallow tree could be controlled by the aril by potentially inhibiting gas and water exchange and



releasing germination inhibiting chemicals (Conway et al. 2002; Meyer 2011; Bower 2009; Renne et al. 2001; Shu-xian et al. 2011).

Germination does occur at low levels in the presence of the aril but several studies have indicated physical removal by both natural and simulated bird digestion increases germination (Bower 2009; Renne et al. 2001; Shu-xian 2012). However conflicting results by Conway et al. (2002) showed aril removal by bird scraping had no such effect. Soaking seeds with intact arils increases germination although it is unclear if germination is achieved through leaching of inhibiting chemicals or a physical breakdown of the aril. Soaking could also allow for increased moisture absorption (Bower 2009; Conway et al. 2000; Samuels 2004). Though these studies have established that the aril does have a limiting effect on seed germination, all studies have failed to address the timing of aril and seed removal which can also potentially have a strong influence on seed germination of Chinese tallow tree seed (Ohkawara 2005; Chavarria 1986). Cameron et al. (2000) showed that seed germination reached maximum levels after 2 years of cold dry storage indicating that seeds could require a period of after ripening to reach full germination potential.

The objectives of this study were to determine: 1) seed bank longevity ; 2) seedling emergence patterns under natural conditions or under simulated natural conditions using fluctuating spring temperatures; 3) evaluate seed germination and viability as a function of time post capsule split (within 1 week of observing); and 4) evaluate the influence of the waxy aril on seed germination. The goal of objective 1 was to refine post treatment site monitoring recommendations for land managers while objective 3 was an effort to determine if length of time attached to the tree and time

post-harvest had any influence on germination or seed viability over time. In addition x-ray analysis was used to determine seed-fill characteristics of Chinese tallow tree seed.

## **Materials and Methods**

### **Emergence Timing**

A single parameter study was established at Paynes Prairie Preserve State Park in Gainesville, FL (29°36'49.16"N, 82°20'04.19"W) to evaluate seedling emergence timing and field seed bank longevity of Chinese tallow tree seed. Trials were initiated before seedling emergence in January 2014 and February 2015 and at a second site in Jay, Florida in March 2015. Both sites contain fully mature, seed-bearing Chinese tallow tree populations. The Paynes Prairie site is categorized as a wet prairie surrounded by basin marsh (Anon. 2014 c), but has converted into a hardwood hammock with patchy canopy cover, due to land use and monitoring changes which have allowed Chinese tallow tree and other woody tree species to move into the area (A. Christman, pers. comm.). This site has traces of species which once probably dominated the area such as pickerelweed (*Pontederia cordata*), maidencane (*Panicum hemitomon*) and other grass and sedge species but now is primarily dominated by Chinese tallow tree and southern blackberry (*Rubus argutus*) with few scattered, sweetgum (*Liquidambar styracifula*) red maple (*Acer rubrum*) and persimmon (*Diospyros virginiana*) trees. The second site, located near Jay, FL (30°45'52.31"N, 87°02'27.60"W), in the western panhandle region, was adjacent to row crop fields and consisted of a cypress (*Taxodium distichum*) dominated wetland with associated sweetgum (*Liquidambar styracifula*) and Chinese tallow trees.

At each site seed rain was excluded from 1 m<sup>2</sup> plots using wooden frames covered with screens to prevent additional seed deposition. Each trial had 10 replications with each frame considered a single replication. All frames were placed at random locations throughout each site where there was evidence of seeds being deposited on the ground. Seed frames were monitored monthly for seedling emergence and emerged seedlings were counted and removed during each visit. Any litter that accumulated on top of the screens was removed at each visit. Emergence data was collected for one year for the 2015 studies and over 2 years for the studies initiated in 2014. Analysis of variances were analyzed in SAS 9.4 using the PROC GLM command to perform analysis between sites and years, separating differences using Fisher's LSD at a significance level of  $\alpha=0.05$ . Further analysis was done using the non-parametric Wilcoxon's sums rank tests using the command PROC NPAR1WAY to determine differences between specific months.

### **Collection Protocol**

Seed for the Chinese tallow tree germination studies were collected from Paynes Prairie Preserve State Park. Seeds were harvested a total of 4 times each collection year (2014 and 2015) beginning within 2 weeks of observed capsule split, which occurred in late October, and were collected from a minimum of 10 trees. Seed containing capsules were harvested directly from the upper tree canopy using a tree pruner, with a minimum of 75 capsules harvested per tree at each collection timing. Capsules from each tree were kept separate and stored in a climate controlled room maintained at  $22^{\circ}\text{C} \pm 3^{\circ}\text{C}$ . Seeds were removed from capsules and sorted within 10 days, removing damaged and immature seeds (contained in green capsules), and 100

seeds from each tree were randomly selected and combined into a bulk sample, from which 100 seeds were randomly selected for each seed treatment.

### **Germination Protocol**

Each individual seed selected for germination was considered an experimental unit (100 replicates). Seeds were planted, in randomly selected cell locations, in a deep cell plug tray<sup>1</sup> filled with potting media<sup>2</sup>. Seeds were planted 1 cm below the soil surface, since this is the depth seeds were commonly found at Paynes Prairie (Wheeler et al. 2014), and burial has proven to increase the likelihood of germination (Renne et al. 2001). After planting, seeds were surfaced watered and then kept moist using subsurface irrigation. Seeds were germinated in a controlled-environment growth chamber under a 9 hr light cycle ( $177 \mu\text{mol s}^{-1} \text{m}^{-2}$  photosynthetic photon flux density [PPDF]) at alternating temperatures of 27C day/15C night. These conditions are associated with spring time daily temperature fluxes in central Florida (Pérez et al. 2009), which correlated with germination noted in the field from a seedling emergence timing study. Germination was checked daily for 60 d, with seeds being considered germinated once the cotyledons were fully visible above the soil surface, at which time, the date was noted and seedling removed from the cell. Seeds that did not germinate after 60 d were tested for viability according to the procedures outlined in the Handbook on Tetrazolium Testing (Moore 1985). The aril was removed from all non-germinated seeds, the seed then was bisected longitudinally and placed into 2 ml micro centrifuge tubes with 0.25 ml of 1% tetrazolium chloride<sup>3</sup> solution. Seeds were allowed to soak for 48 hours in the

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<sup>1</sup> 200 Cell Plug Tray, TO Plastics®, 830 County Road 75-PO Box 37-Clearwater, MN 55320

<sup>2</sup> Fafard 4 Mix, Sungro Horticulture®, 770 Silver Street Agawam, MA 01001

<sup>3</sup> 2,3,5-triphenyltetrazolium chloride (TZ), Sigma-Aldrich Life Science, St. Louis, MO

dark at 25C, removed and tested visually for viability. Seeds were considered viable with firm endosperm tissue with a dark pink-red stained embryo. Statistical analysis was performed using time event survival analysis to account for left (seeds that are lost during the study) and right censoring (seeds which would have germinated during the study but did not germinate before study termination) of seeds. This technique is more accurate at representing germination timing due to censored observations underestimating means and variances invalidating ANOVA results (Pérez & Kettner 2013; Scott et al. 1984; McNair et al. 2012).

### **On Tree Seed Storage**

To determine the effect of on tree seed storage, seed germination and viability was evaluated as a function of time of removal after capsule split (see harvest timings under collection section). A total of 4 harvests were conducted with initial harvest occurring at the start of seed capsule split, in mid-October, and then re-occurred every 2 weeks for a total of 8 weeks. Harvests were initiated October 31 and terminated December 12; and October 22 and terminated December 21<sup>st</sup> for 2014 and 2015, respectively. Seeds from each harvest interval were planted within 10 days of each harvest, with harvest considered a treatment effect. Seed germination and viability were determined utilizing methodology outlined in the germination protocol section. Both trials had 4 harvest treatments. Seed collection and other environmental conditions were the same as previously stated in the seed collection and germination protocols.

### **Off Tree Seed Storage**

To evaluate the effect of harvest timing and storage conditions, seeds from each harvest in 2014 were placed at 5 C or 20 C for 6 months after each harvest interval and

evaluated for germination and viability according to procedures outlined previously. Stored seeds were compared to controls that were germinated within 10 days of harvest.

### **Aril Coating**

To evaluate the effect of the aril on Chinese tallow tree seed germination, seeds from each harvest in 2015 (see collection protocol section) were fully submerged in 250 ml of distilled water for 48 hours. Seeds were gently stirred 2X daily to prevent mold growth, but not aggressively enough to visibly damage the seed. After soaking, the aril was either removed or left intact. Soaking allowed the removal of the waxy aril with minimum impact to the hard seed coat underneath and also simulated semi-natural conditions that might be experienced during dispersal. Non-soaked seeds were included as a control and seed germination and viability were evaluated as detailed in previous sections.

### **Seed Fill**

To evaluate the characteristics of Chinese tallow tree seed fill, 100 seeds were selected from the bulk sample of each harvest in 2015. These seeds were sent to The Ohio State University Ornamental Plant Germplasm Center<sup>4</sup> within the Horticulture and Crop Science Department for X-ray analysis. Seed from each harvest were visually evaluated, categorizing seeds as filled or compromised (empty, mal-malformed, desiccated, predation) as well as visually estimating the fill of each seed on a scale of 0-100%, 100% representing completely filled seed and 0% representing empty seed (Figure 3-1).

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<sup>4</sup> The Ohio State University Ornamental Plant Germplasm Center, 670 Vernon L. Tharp Street, Columbus, OH 43210, <http://opgc.osu.edu/>

## **Statistical Analyses**

Due to a sampling error that occurred in 2014, harvest 1 was removed from any analysis which included 2014 data. Analysis of germination time-to-event and categorical data were performed using SAS software (v. 9.4, SAS, Inc., Cary, NC, USA.), with germination as the event of interest. Seeds that germinated during the 60 day experiment duration were coded as 1. Seeds that did not germinate by day 60 were coded as 0. No seeds were lost due to random censoring (Pérez & Kettner 2013). Germination parameters- and Kaplan-Meier estimates of survivor functions were generated using PROC LIFETEST. Survivor functions were stratified by treatments evaluated for each study. The null hypothesis that survivor functions were the same within treatments was tested by calculating the log-rank statistic. Subsequently, Cox regression was performed using the PROC PHREG statement. Analysis of Martingale and Schoenfeld residuals was conducted as described in Pérez & Kettner (2013). Extensions to the Cox model were done when necessary, adding a time depended covariate to accommodate those instances when non-proportionality occurred, after which we repeated the model building procedure in PROC PHREG. The Cox model or extended model was then used to obtain the effect of harvest date, storage and seed coat treatments on germination for any day within the 60 day observational period. Cox models for each study are presented in Table 3-1. Orthogonal linear contrasts were used to test the null hypothesis of similar slope coefficients for each treatment (Pérez & Kettner 2013). These are presented in Tables 3-2 and 3-3 for the storage and aril removal studies, respectively.

Pearson's chi-square tests ( $Q_p$ ) were used to statistically evaluate categorical values of the components of seed viability. Simple comparisons of viability components, were executed in Excel 2013 (Microsoft Inc., Redmond, WA, USA) using the conservative methods described by Sheskin (2011). Significance was evaluated at  $\alpha=0.05$  for ( $Q_p$ ) and at  $\alpha=0.025$  and  $0.005$  for simple comparisons. These more conservative values were used for the simple comparisons in order to reduce the likelihood of committing Type I errors that may be associated with any pair of comparisons at the  $\alpha=0.05$  level (Sheskin 2011; Pérez & Kettner 2013). Standardized residuals were calculated to identify which cell frequencies were major contributors to significant  $\chi^2$  values. Standardized residuals were evaluated at  $\alpha=0.05$  and  $0.01$ . Seed fill was analyzed using independent, 2 sample t-tests run in SAS 9.4 to evaluate sample fill means of each harvests at an  $\alpha=0.05$ .

## **Results and Discussion**

### **Emergence Timing**

Studies were established at two locations to monitor seedling emergence over time from seed deposits under established mature trees. The objectives were to: 1) determine when tallow seeds emerge during the season, 2) if differences occurred across locations and 3) correlate field emergence with seed physiology studies.

Chinese tallow tree seedling emergence, in the first year of monitoring, occurred from March-June at the Paynes Prairie site. Emergence peaked in April for both plots established in 2014 and 2015 (Table 3-4). No significant difference in emergence ( $P>0.05$ ) was detected between the 2014 and 2015 seed exclusion plots at the Paynes Prairie site indicating similar site conditions during both years of seedling emergence monitoring. A similar trend in emergence was apparent at the Jay, Florida site but with



seedling emergence initiating 1 month later from April-July, with peak emergence occurring in May (Table 3-4).

Seed plots established at Paynes Prairie in 2014 were also monitored in 2015 and indicated a decline ( $P < 0.05$ ) in seedling emergence after 2 growing seasons. Unlike 2014, emergence was confined to 3 months, with an average of 3.1, 0.3, and 0.1 seedlings emerging across the plots in April, May, and June respectively. Similar to the single year studies, emergence was significantly higher in April 2015 but declined by 81.67% when compared to April 2014 seedling emergence.

These results indicate seedling emergence timing varies by region with emergence at northern sites possibly delayed by a lag in seasonal warming or other climatic factor. The seasonal cycling of seedling emergence and regional variations in emergence timing also suggest Chinese tallow tree seeds possess a form of enforced and/or physiological dormancy. Specific environmental conditions could include proper oscillating temperatures to trigger germination but could also require specific changes in the seed coat or aril to occur to further release the seed (Lambers et al. 2008). Future research should incorporate more intensive site monitoring of temperature, rain fall, soil and light conditions to allow for a clearer understanding of the conditions at each site and also monitor a known number of seeds to further understand seed longevity.

### **On Tree Seed Storage**

No significant effect of harvest timing was observed on the viability and germination (Table 3-5) of Chinese tallow tree seeds collected in 2014 and 2015 with no differences detected between years ( $P = 0.0660$ ) (Table 3-1). Slightly more than 50% of the seeds were viable, with greater viability in 2015. Germination frequency was greater in 2014, with 27% of the viable seed fraction germinating within the 60 day

germination period. Germination occurred on average 35-40 days after planting. These studies were conducted on unaltered seed, with the aril left intact, and this structure may be influencing germination. Our results indicate that when the seed is harvested directly from the tree and placed under ideal conditions within 10 days, seeds have limited germination indicating some level of dormancy. It is interesting that seed germination characteristics were not influenced by the length of time attached to the mother tree, indicating that dormancy was not changing, neither increasing nor decreasing. However, our results should be interpreted with caution, due to the occurrence of extremely large 95% Wald Confidence Limits.

### **Off Tree Seed Storage**

Seeds from the individual harvests from 2014 were stored for 6 months at ambient (22C) or cold (5C) conditions and subsequent tests of seed viability and germination were performed on each lot. There were no differences between harvest nor an interaction between storage treatment in terms of viability, so results were pooled across harvests. Significant differences were observed between seeds that were immediately germinated compared to seeds stored for 6 months at 5 or 20 C and there were no differences between the two temperature treatments (Table 3-6). Immediately germinated seeds were approximately 4 times more likely to germinate within 60 days than seeds stored for 6 m at ambient conditions or 5C while fresh seeds also took significantly longer to germinate, germinating approximately after 10 days seeds stored for 6 months in either temperature treatment.

These results are similar to those reported by Renee et al. (2001) where they observed a higher percentage of emergence in fresh seeds vs. stored seeds (75.3 and 44.5% respectively;  $P=0.0001$ ). A possible explanation for the difference in germination

observed in stored seeds is that the aril, after 6 m of storage, might gradually harden due to the relatively dry storage conditions. As the aril hardens it possibly seals the seed, preventing water absorption and becoming physical mechanism inhibiting germination of a non-dormant seed (Candiani et al. 2004; Finch-Savage & Leubner-Metzger 2006). In contrast, dry storage conditions might result in compromised or cracked seeds during the drying/hardening process. Therefore, seeds with compromised arils might be able to draw water more easily into the hard seed coat beneath, explaining the earlier mean germination timing observed for stored seeds that germinated.

Dormancy of Chinese tallow tree seeds is further supported after analyzing seed viability components. Germination decreased when seeds were stored, but there was no significant difference in the total viable seed fraction 6 m after harvest, indicating seed viability is not being loss overtime and in a dormant state. While this study indicates dormancy it is not conclusive within these context; more testing is required specifically looking at germination capacity of the excised embryo, water uptake, aril characteristics over time and other variables that might trigger germination in Chinese tallow tree seeds. Future experiments should also increase the number of seeds tested to account for the variability within the seed lots collected.

### **Aril Coating**

A significant difference in germination was detected for harvest date ( $P=0.0079$ ) and aril treatments ( $P=0.0014$ ), with no significant interactions or differences in viability components observed for harvest or treatment (Table 3-1).

When comparing aril treatments within harvest, no significant difference in germination was found between the unsoaked seed (6-14% of viable seed germinating) and seeds soaked for 48h with an intact aril (2-7% of viable seed germinating) (Table 3-7). Seeds soaked with the aril removed showed significantly higher germination (32-66% of viable seed germinating), resulting in up to 8-fold greater germination than seeds soaked with an intact aril and 31-fold greater germination compared to unsoaked intact seeds. These results indicate that the aril is significantly effecting seed germination, possibly providing a form of innate dormancy. Aril removal at different intervals also significantly influenced germination between harvests where a significant increase in germination was observed at harvest 4, which was 2.1 times more likely to germinate than harvest 1 and 1.7 times more likely to germinate than harvest 2, for every day spent on the tree (Table 3-6).

It appears the longer seeds were present on the tree with the aril intact, the more likely they were to germinate so long as the aril was subsequently removed. Similar results were found by Renne et al. (2009), where seeds that been defecated by a bird showed significantly greater germination compared to non-bird-fed seeds. This also indicates there might indeed be some form of after ripening occurring with its effect masked by the presence of the aril. This enhancement of germination overtime could also be the result of the saprophytic black mold of the genus *Pullularia* spp. which is commonly found on the aril of Chinese tallow tree seeds (Scheld et al. 1980; Burns and Miller 2004). Research shows within 8 weeks the mold turns the white tallow a dark black as it grows and feeds on the aril, eventually penetrating the hard seed coat and ultimately resulting in embryo mortality (Figure 3-2) (Bruce 1993).

Due to no significant difference between viability components across all harvests within this seed treatment, seeds appear to benefit from the fungal interaction between 6 and 8 weeks after capsule split. However it is unknown how long the benefits are maintained before seed losses begin to occur since no significant losses were observed within the timing of this study. This suggests the gradual breakdown from the fungus of the aril and/or hard seed coat, at earlier stages, could enhance seed germination (Chavarria 1986). Fungal development could also be beneficial; since there is evidence of symbiotic relationships developing between seeds. For example, many species of orchids require a fungus to support embryo growth (Atwell et al. 1999). Therefore, mold growth could have caused the after ripening effect which enhanced seed germination over time, while also effectively masking any after ripening benefits until after its removal. Mold growth also might also be responsible to some extent for 27% of the compromised seeds collected directly from the tree.

### **Seed Fill**

No significant differences between harvests means of seeds collected in 2015 were detected when evaluating seed fill using visual estimates ranging from 0-100% (data not shown). Based on pooled data of all harvests, 27% of all seeds collected directly from the tree are compromised in some respect. Seeds were either immature, empty, or predated. These data indicate the presence of compromised seed/a lack of seed fill account for 72% of the nonviable seed observed in the 2015 germination experiments.

Collectively, these studies indicate that enforced dormancy is most likely occurring since emergence only occurs at a certain times of the year - indicating specific germination conditions. Seeds appear to maintain viability after 6 months under dry

storage conditions, regardless of temperature treatment. However, in the field, seedling emergence was reduced by 82-100% at peak emergence times after a single year indicating the seed bank might be short lived (<3 years) under our conditions. These studies also indicate the aril greatly influences the fate of each Chinese tallow tree seed, possibly as a physical barrier to water and/or gases. However, the interaction of the fungal pathogen *Pullararia* remains questionable and should be investigated further. These studies cannot conclusively state what form/or if dormancy is occurring but do provide incentive for more detail oriented research focusing on aril characteristics over time and how gas and water exchange is affected by changes to the aril. Future research also should include collecting seeds via seed rain as a function of capsule split providing a more accurate description of what happens to seeds if they are dispersed by natural barochory (dispersal by gravity).

Table 3-1. Summary table of extended Cox models for Chinese tallow tree (*Triadica sebifera*) seed germination exposed to different seed treatments and germinated for 60 days

Covariate( $x_i$ )	Parameter estimate, $\beta_i$	SE <sup>2</sup> of $\beta_i$	$\chi^2$	$Pr > \chi^2$	Hazard ratio
<u>On Tree Storage</u>					
harvest <sup>3</sup>	0.16438	0.31322	0.2754	0.5997	1.179
year	2.50641	1.36358	3.3786	0.066	12.261
year x harvest	-0.0238	0.2203	0.0117	0.914	0.976
year x days	-0.08304	0.03305	6.3137	0.012	0.92
<u>Off Tree Storage</u>					
harvest <sup>4</sup>	0.0784	0.19123	0.1681	0.6818	1.082
storage <sup>5</sup>	1.11056	1.19333	0.8661	0.352	3.036
harvest x storage	0.37611	0.219	2.9495	0.0859	1.457
storage x days	-0.08746	0.02694	10.5408	0.0012	0.916
<u>Aril Removal<sup>6</sup></u>					
harvest	1.03533	0.38986	7.0525	0.0079	2.816
treatment	1.1014	0.34459	10.2161	0.0014	3.008
treatment x harvest	0.05075	0.11764	0.1861	0.6662	1.052
harvests x days	-0.02289	0.00842	7.3805	0.0066	0.977

<sup>1</sup> The extended Cox models for seed treatments have the equation:  $h_o(t) \exp(\beta_1 x_1 + \beta_2 x_2)$  (Pérez & Kettner 2013)

<sup>2</sup> SE denotes standard error.

<sup>3</sup> A total of 4 harvests occurred for each study with harvests initiating 2 weeks after capsule split and then occurring there after every 2 weeks. Fresh seed harvest treatments were used as the control for all other studies, using the seeds collected the year the study was run.

<sup>4</sup> Harvest 1 was excluded due to error.

<sup>5</sup> Six month storage treatments included seeds stored at 5C and ambient temperature vs no storage.

<sup>6</sup> Aril treatments consisted of unsoaked seeds, seeds soaked 48 h in distilled water aril left intact and seeds soaked for 48 h in distilled water with aril removed.

Table 3-2. Orthogonal contrasts for germination of Chinese tallow tree (*Triadica sebifera*) seed collected in 2014 from Paynes Prairie Preserve State Park, comparing storage treatments as a function of harvest timing

Component			Hazard Ratio	95% Wald Confidence Limits	
<u>Harvest comparisons within seed treatment</u>					
Control					
H2 <sup>1</sup>	vs	H3	1.109	[0.506,	2.431]
H2	vs	H4	0.883	[0.420,	1.856]
H3	vs	H4	0.796	[0.373,	1.701]
6 Month @ Ambient					
H2	vs	H3	0.159	[0.019,	1.322]
H2	vs	H4	0.161	[0.019,	1.337]
H3	vs	H4	1.012	[0.326,	3.137]
6 Month @ 5 C					
H2	vs	H3	0.662	[0.111,	3.960]
H2	vs	H4	0.278	[0.058,	1.337]
H3	vs	H4	0.42	[0.109,	1.623]
<u>Seed treatment comparisons within harvests</u>					
Harvest 2					
T0 <sup>2</sup>	vs	T1	13.715	[1.795,	104.771]
T0	vs	T2	6.839	[1.543,	30.308]
T1	vs	T2	0.499	[0.045,	5.496]
Harvest 3					
T0	vs	T1	1.969	[0.739,	5.247]
T0	vs	T2	4.08	[1.151,	14.459]
T1	vs	T2	2.072	[0.518,	8.286]
Harvest 4					
T0	vs	T1	2.502	[0.971,	6.448]
T0	vs	T2	2.151	[0.877,	5.276]
T1	vs	T2	0.86	[0.289,	2.559]

<sup>1</sup> H: Harvest number; Harvests occurred 2 weeks after seed capsule split and occurred every two weeks after the initial harvest. Harvest 1 not included due to error.

<sup>2</sup> T0: Control, no storage, seed subjected to germination within 10 days of harvest T1: 6 month storage at ambient conditions, T2: 6 month storage at 5 C.



Table 3-3. Orthogonal contrasts for germination of Chinese tallow tree (*Triadica sebifera*) seed collected in 2015 from Paynes Prairie Preserve State Park, comparing aril treatments as a function of harvest timing

Component	Hazard Ratio	95% Wald Confidence Limits
<u>Harvest comparisons within seed treatment</u>		
Control		
H1 vs H2	0.551	[0.161, 1.884]
H1 vs H3	0.48	[0.145, 1.594]
H1 vs H4	0.654	[0.185, 2.318]
H2 vs H3	0.87	[0.316, 2.400]
H2 vs H4	1.186	[0.399, 3.529]
H3 vs H4	1.363	[0.473, 3.927]
48 h soak aril intact		
H1 vs H2	0.999	[0.063, 15.977]
H1 vs H3	0.332	[0.035, 3.189]
H1 vs H4	0.245	[0.027, 2.194]
H2 vs H3	0.332	[0.035, 3.191]
H2 vs H4	0.245	[0.027, 2.196]
H3 vs H4	0.739	[0.165, 3.303]
48 h soak aril removed		
H1 vs H2	0.814	[0.467, 1.419]
H1 vs H3	0.61	[0.359, 1.036]
H1 vs H4	0.476	[0.285, 0.795]
H2 vs H3	0.75	[0.452, 1.243]
H2 vs H4	0.585	[0.359, 0.953]
H3 vs H4	0.78	[0.494, 1.232]
<u>Seed treatment comparisons within harvests</u>		
Harvest 1		
T0 vs T1	4.063	[0.454, 36.353]
T0 vs T2	0.161	[0.056, 0.467]
T1 vs T2	0.04	[0.005, 0.294]
Harvest 2		
T0 vs T1	7.363	[0.906, 59.848]
T0 vs T2	0.238	[0.104, 0.547]
T1 vs T2	0.032	[0.004, 0.238]
Harvest 3		
T0 vs T1	2.808	[0.745, 10.584]
T0 vs T2	0.205	[0.095, 0.443]
T1 vs T2	0.073	[0.022, 0.238]
Harvest 4		
T0 vs T1	1.523	[0.430, 5.399]
T0 vs T2	0.117	[0.050, 0.277]
T1 vs T2	0.077	[0.028, 0.216]

<sup>1</sup> H: Harvest number; Harvests occurred 2 weeks after seed capsule split and occurred every two weeks after the initial harvest.

<sup>2</sup> T0: Control: aril intact no soaking, T1: soaked for 48 hours & aril intact, T2: soaked for 48 hours then aril removed

Table 3-4. Monthly seedling emergence of Chinese tallow tree (*Triadica sebifera*) at Paynes Prairie Preserve Florida State Park (PPP), Gainesville, FL and a location near the West Florida Research and Education Center (WFREC), Jay, FL. Combined means and standard errors of 10, 1 m<sup>2</sup> plots.

Month	PPP 2014	PPP 2015	WFREC 2015
January	0	0	0
February	0	0	0
March	4.1 ± 1.8	2.7 ± 1.3	0
April	16.6 ± 9.0*	13.7 ± 7.3*	8.6 ± 1.8
May	0.6 ± 0.5	0.5 ± 0.2	37.9 ± 12.9*
June	0.2 ± 0.1	0.1 ± 0.1	12.9 ± 8.4
July	0.1 ± 0.1	0.2 ± 0.1	0.1 ± 0.1
August	0.1 ± 0.1	0	0.2 ± 0.1
September	0	0	0
October	0	0	0
November	0	0	0
December	0.1 ± 0.1	0	0
LSD <sub>0.05</sub>	7.5	5.9	14.5

\* Significantly different from all other values in column determined using ANOVA with differences determined using Fisher's LSD at alpha = 0.05.

Table 3-5. Germination (% germination of viable seed) of Chinese tallow tree (*Triadica sebifera*) seeds collected over 3 harvest timings at Paynes Prairie Preserve State Park, Gainesville, FL in 2014 and 2015

Harvest	Treatment	
	2014	2015
Weeks 4 <sup>1</sup>	13 (26) <sup>2</sup>	7 (12)
Weeks 6	12 (29)	8 (14)
Weeks 8	15 (31)	6 (10)

<sup>1</sup> Harvests were initiated 2 weeks after seed capsule spilt and occurred every 2 weeks thereafter for 2 harvests events. Each harvest treatment consisted of 100 seeds. Data for week 2 not included due to sampling error.

<sup>2</sup> % viable seed (sum of germinated and tetrazolium viable seed).

Table 3-6. Germination (% germination of viable seed) for Chinese tallow tree (*Triadica sebifera*) seeds collected over 3 harvest timings at Paynes Prairie Preserve State Park, Gainesville, FL in 2014

Harvest	Treatment					
	Fresh		6 Months 22 C		6 Months 5 C	
Weeks 4 <sup>1</sup>	13	(26) <sup>2</sup>	2	( 4)	1	( 2)
Weeks 6	12	(29)	3	( 6)	6	(11)
Weeks 8	15	(31)	7	(13)	6	(11)
Pooled Means <sup>3</sup>	13 A <sup>4</sup>	± 1.5	4 B	± 2.7	4 B	± 3.0

<sup>1</sup> Harvests were initiated 2 weeks after seed capsule spilt and occurred every 2 weeks thereafter for 3 harvests events. Each harvest treatment consisted of 100 seeds. Data for week 2 not included due to sampling error.

<sup>2</sup> % viable seed (sum of germinated and tetrazolium viable seed).

<sup>3</sup> Means and standard errors of pooled harvests within storage treatment.

<sup>4</sup> Capital letters denote significant differences (P<0.05) between treatments, within the same row, obtained through the use of cox regression analysis.

Table 3-7. Germination (% germination of viable seed) for Chinese tallow tree (*Triadica sebifera*) seeds collected over 4 harvest timings at Paynes Prairie Preserve State Park, Gainesville, FL in 2015

Harvest	Treatment					
	No Soak		48 Hour Soak		Aril Removed	
Weeks 2 <sup>1</sup>	6	A <sup>2</sup> (72) <sup>3</sup>	2	A (65)	32	B a (73)
Weeks 4	12	A (59)	2	A (63)	40	B a (68)
Weeks 6	14	A (57)	5	A (63)	57	B ab (60)
Weeks 8	10	A (59)	7	A (58)	66	B b (61)

<sup>1</sup> Harvests were initiated 2 weeks after seed capsule split and occurred every 2 weeks thereafter for 4 harvests events. Each harvest treatment consisted of 100 seeds.

<sup>2</sup> Capital letters denote significant differences ( $P < 0.5$ ) between treatment within a harvest timing, and lowercase letter denote differences between harvest timing within treatment obtained through the use of cox regression analysis.

<sup>3</sup> % viable seed (sum of germinated and tetrazolium viable seed).

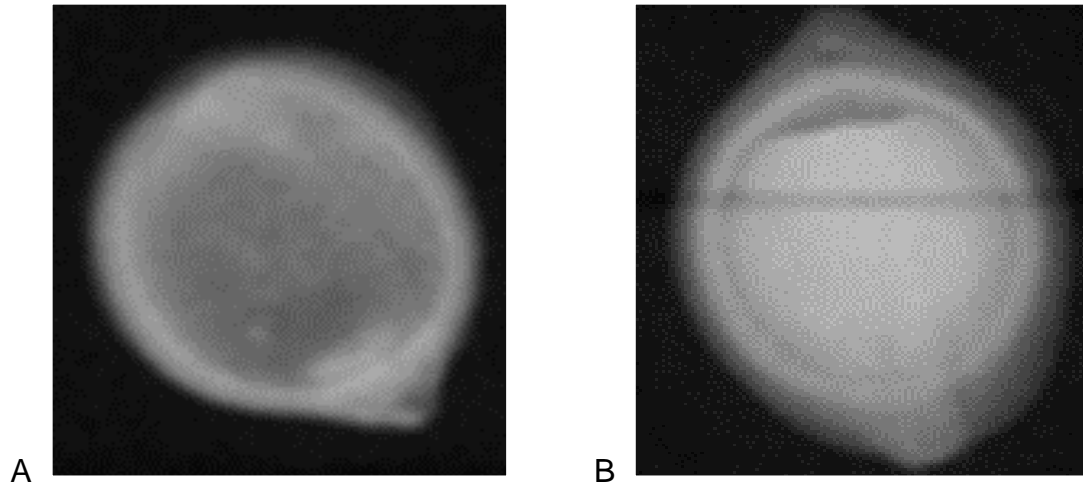


Figure 3-1. Rating scale used to determine seed fill characteristics of Chinese tallow tree (*Triadica sebifera*) using seed x-rays. A) Empty; seed rated as 0% seed fill and B) Filled; seed rated as 100% seed fill

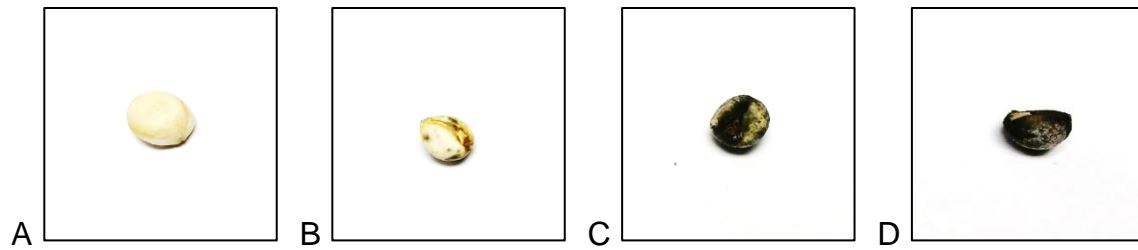


Figure 3-2. Progression of a black mold growth on Chinese tallow tree (*Triadica sebifera*) seeds harvested directly from adult trees at Paynes Prairie Preserve State Park, Gainesville, FL in fall of 2015, after capsule split over an 8 week period. A) Seed harvested October 12; 2 weeks after capsule split, B) Seed harvested October 28th; 4 weeks after capsule split, C) Seed harvested November 20th; 6 weeks after capsule split and D) Seed harvested November 29th; 8 weeks after capsule split

## CHAPTER 4 CONCLUSIONS

Chinese tallow tree, an invasive species from Southeast Asia, has proven to be a difficult species to manage. Since its introduction in the mid 1700's this species has gradually spread throughout the Southeastern United States causing economic and environmental harm. This species is known to displace native and agronomic species, transform environments and alter ecosystem processes by creating monotypic tree stands within approximately 20 years.

Management of this highly prolific, fast growing tree species currently relies heavily on chemical control. Studies have shown that current standards for broadleaf tree control do not work as effectively on Chinese tallow tree as on other species. Herbicide recommendations often require repeat applications to address failed treatments and aggressive regrowth on Chinese tallow tree and much of the literature fails to include recently developed herbicides. Therefore, we compared the effectiveness of aminocyclopyrachlor, glyphosate, hexazinone, imazapyr and triclopyr ester when used as a basal, hack and squirt or cut stump herbicide application to determine which herbicides and application methods provided the most effective control options for Chinese tallow tree.

Aminocyclopyrachlor affectively controlled Chinese tallow tree when applied using basal bark, hack and squirt and cut stump application methods. The combination treatments of aminocyclopyrachlor and imazapyr also provided similar results as treatments of aminocyclopyrachlor alone. Imazapyr provided similar control as aminocyclopyrachlor if used in hack and squirt and cut stump applications but was not effective at controlling Chinese tallow tree when applied basally. Triclopyr, a highly



recommended commercial standard, did not provide consistent control over all application methods. Glyphosate and hexazinone both failed to provide adequate control of Chinese tallow tree in hack and squirt and cut stump treatments. Hydrologic manipulation and site flooding when used in combination with herbicides shows potential as a management tool for Chinese tallow tree control. Prolonged site flooding occurred at the WFREC site and appeared to enhance the effectiveness of all herbicide treatments at that site and warrants further study.

After adult trees are controlled, managed sites are still vulnerable to recolonization via seeds. Therefore, understanding Chinese tallow tree seed biology is necessary for long-term management. Field tests were conducted to determine seedling emergence patterns and potential seed bank longevity. Chinese tallow tree seedling emergence under field conditions was shown to occur over a 3 month period during spring. Seedling emergence peaked in April for the Gainesville location and in May for the Jay location. Seedling emergence was observed to follow the same pattern of emergence across locations. Following spring time emergence peaks, seed germination ceases until the following year, indicating some form of dormancy. Seedling emergence after 2 growing seasons declined significantly with 50-100% reductions observed in some plots, supporting the hypothesis that Chinese tallow tree seed banks may only last 2-3 years after removal of seed sources.

Growth chamber germination and viability testing were performed on seeds collected at 2 week intervals, after capsule split, at Paynes Prairie Preserve Florida State Park in Gainesville, FL in 2014 and 2015. Harvest timing, the effect of 6 month storage and the timing of aril removal were evaluated for seed germination and viability.

Harvest timing had no significant effect on seed germination or viability if the aril remained intact. Intact Chinese tallow tree seed stored at 5 C or ambient temperature for 6 months were 74-76% less likely to germinate than fresh seeds. Seeds that did successfully germinate also did so 10 days sooner than fresh seed, leading to the hypothesis that the aril potentially hardened due to dry storage conditions causing the aril to act as a barrier to water and gas exchange. Any seeds that did germinate most likely did so due to their arils being compromised.

The aril appears to have a masking effect on harvest timing since seed germination did increase with time if the aril was removed at harvest. Complete aril removal resulted in a significant reduction in the average amount of time required for seed germination as well as causing an increase of 85% in seed germination. The timing of aril removal also increased seed germination the longer the aril was maintained on the seed after capsule split. Since the presence of a black mold becomes more prevalent on the aril over time, it is suspected that there could be a beneficial interaction of the mold on seed germination.

Seed viability was similar over all treatments in 2014 and 2015. X-ray seed fill analysis showed that 72% of all nonviable seed in 2015 had <100% seed fill further supporting the results observed during viability testing. Since viability was constant and seed loss was minimal even after 6 months of storage it is suspected that Chinese tallow tree seeds do experience dormancy.

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## BIOGRAPHICAL SKETCH

Heather VanHeuveln was raised in Biscayne Park, Florida. She is the second oldest of 4 daughters and is the first to achieve a higher level education degree. She graduated from the Biomedical and Environmental Advancement Magnet at North Miami Beach Senior High School in 2005 and then attended the University of Florida, where she earned a Bachelor of Science, graduating Cum Laude, in environmental science in 2009.

In February 2012, Heather began work as a Florida State Parks AmeriCorps member at Paynes Prairie Preserve State Park. Under the guidance of park biologist Andrea Christman, she was trained to be an invasive plant management technician as well as a trained wildland firefighter. In December of 2012 upon completion of her AmeriCorps year of service, Heather was hired by Amy Richards and Dr. William Haller at UF/IFAS Center for Aquatic and Invasive Plants as a biologist/project assistant. There she assisted with invasive aquatic plant management research and provided project support to the information office in regards to their invasive plant education intuitive.

In January 2014, Heather began graduate studies in agronomy. As a graduate student, Heather won 3<sup>rd</sup> place in the Florida Exotic Plant Pest Councils Annual Graduate Student Oral Presentation Competition in 2015 and was nominated to be inducted into Gamma Sigma Delta. She served as a graduate Research Assistant to Dr. Gregory MacDonald and served as a Teaching Assistant for PLS4601, Principles of Weed Science.

Heather received her master's degree from the University of Florida in summer of 2016.