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Potential for introduced-range expansion of Chinese tallow tree (Triadica sebifera) in the southeastern United States

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POTENTIAL FOR INTRODUCED-RANGE EXPANSION OF CHINESE TALLOW TREE (*TRIADICA SEBIFERA*) IN THE SOUTHEASTERN UNITED STATES

A Thesis
Presented to
the Graduate School of
Clemson University

In Partial Fulfillment
of the Requirements for the Degree
Master of Science
Plant and Environmental Science

by
Isaac Park
May 2009

Accepted by:

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ABSTRACT

The potential for populations of invasive plants to differ in their response to stressful environmental conditions or in their invasiveness is an underexplored issue in determining introduced species’ range limits. Introduced genotypes might differ in their response to freezing temperatures, soil type, or differing biotic factors within their introduced range. We examined the potential of Chinese tallow tree seeds (*Triadica sebifera* (L.) Small) collected from two genetically distinct areas of its introduced range in the United States (North Carolina and South Carolina) as well as from the northern and southern portions of its native range in China to germinate and for seedlings to survive winter conditions inland of the species’ current coastal distribution in the state of South Carolina. Germination success of seeds from these four source populations was compared between fall plantings that mimic natural dispersal timing and spring plantings after the last frost in areas within (coastal sites) and inland (midland and piedmont areas) of its current distribution in South Carolina. In separate studies, overwinter survival, stem damage, budbreak timing and response to freeze events occurring before and after budbreak were compared among seedlings of the four source populations. Overall, seed germination success was lower in the colder piedmont areas than the warmer coastal sites, and seeds and seedlings from South Carolina genotypes showed greater reduction in germination success by inland winters, greater winter damage and reduced budbreak success in the field, and lower survival after prolonged freeze events than genotypes from North Carolina or China. These results show that genetically determined differences in environmental constraints exist among introduced genotypes of Chinese tallow, and that the North Carolina genotype shows greater potential for inland expansion than the South Carolina one. These population differences must be taken into account when evaluating the potential range expansion of Chinese tallow.
ACKNOWLEDGEMENTS

I would like to thank my advisor Saara DeWalt for all of her help at every stage of this thesis; the Nemours Wildlife Foundation and the Belle W. Baruch Wildlife Foundation for access to their facilities; John Mueller, Bill Box, James Rushing, T.J. Savereno, and Austin Jenkins for their assistance in locating appropriate field sites; Douglas Bielenberg and David Tonkyn for guidance on design of the experiments; W. Rogers and E. Siemann, and J. Zou for providing seeds in China; William Bridges and Patrick Gerard for statistical advice; Kalan Ickes, Danielle Zoellner, Carrie Woods, and Ben Taylor for suggestions that improved this manuscript; and NSF grant DEB-0315796 to ES and WER for funding to collect seeds in China.
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CHAPTER ONE
INTRODUCTION

Because of growing interest in predicting the potential effects of invasion by exotic plant species, as well as the potential for range shifting posed by global climate change, understanding the factors that limit species’ ranges is a subject of major importance in population biology. However, many interacting biotic and abiotic factors may determine the range limit of a species, such that determining range limits is often difficult. Exotic, invasive species often have not expanded to fill their potential invasive ranges as predicted by models of range limitation that include modern changes in climate (Burkett et al. 2005; Parmesan et al. 2005). Climate generally is considered the predominant factor determining the range of most plant species (Woodward 1987), particularly in the portions of a range that are greatest in latitude, highest in elevation, most xeric, or otherwise most subject to climatic extremes (Loehle 1998; Nobel and Smith 1983; Richardson and Bond 1991; Woodward 1987). Other factors such as seed dispersal, herbivory, and parasitism can also affect species ranges (Case et al. 2005; Primack and Miao 1992), but are considered secondary in importance in determining the gross-scale range.

Cold temperatures may limit a plant species’ range through frost damage to tissues, cold-induced cavitation of xylem vessels (Miller-Rushing and Primack 2008; Nardini et al. 2000), or reduction in the germination success of overwintering seeds (Brandt et al. 2004). In some cases, even extremely small changes in minimum
temperature have been found to have population-level effects at the northern edges of populations (Nobel 1980). A species’ range may also be restricted to locations where the duration of moderate temperatures is enough for sufficient growth to occur to compensate for damage accrued over the rest of the year and still generate enough new growth to prevent overtopping or competitive exclusion by other species (Kullman 1993). The growing season must also be long enough for fertilization to be successfully completed (Pigott and Huntley 1981), and for the maturation and accumulation of nutrient reserves in seeds (Galen and Stanton 1993). Species may be excluded from areas in which temperatures remain too low for seeds to break dormancy or germinate until late in the year. Delayed germination can result in germination during an unfavorable time of year (Marshall 1968; Woodward et al. 1990) or a reduction in the ability of seedlings to compete with other species that germinate earlier in the season (Gross and Smith 1991).

Determining the potential range of an exotic invasive plant involves all the complexities required to determine the species’ native range, but also introduces additional considerations due to the possibilities of founder effects on the invasive phenotype or differing selective pressure between native and invasive populations. In many cases, the invasive ranges of species have been predicted through describing the climatic conditions that contain the native range, and determining what areas within the possible invasive range fit within that climate envelope (Peterson 2003). However, it has been shown that different populations may have different germination requirements across the species’ range (Donohue et al. 2005a), that there can be strong natural selection for different germination parameters (Donohue 2002; Wagner and Simons
2008), and that such selection is powerful enough to act as a filter against genotypes with unfavorable germination timing (Donohue et al. 2005c). Thus, it is likely that there may be different temperature, day length, and moisture conditions required to stimulate germination (germination requirements) of genotypes found in different areas of a species’ range.

Chinese tallow tree, *Triadica sebifera* (L.) Small (Euphorbiaceae; previously known as *Sapium sebiferum*), is an invasive species of Chinese origin in the United States (U.S.). In the southeastern U.S., Chinese tallow has naturalized along the Gulf Coast and East Coast from Texas to North Carolina (Fig. 1) and has become a dominant species in coastal ecosystems (Bruce et al. 1997). It is found in scattered, small populations in non-coastal areas, but has not become abundant in these areas. Chinese tallow tree is found on a variety of soil types, including saline soils and both acidic and alkaline soils (Barrilleaux and Grace 2000; Bruce 1993; Draper 1982) and is believed to be restricted primarily by frigid or arid conditions (Draper 1982; Jubinsky and Anderson 1996; Renne 1996). The capability of Chinese tallow to expand its range into new regions of the U.S., particularly inland and more northern locations, is largely unknown (Pattison and Mack 2008), although there is evidence that it may be capable of expansion well beyond its current range (Pattison and Mack 2009).

In its native range, Chinese tallow grows in colder climates than those in which it is currently found in the U.S. (Lee 1956). Estimates of Chinese tallow’s potential invasive range were made using the climate model CLIMEX (Pattison and Mack 2008), which matched moisture, temperature, and other climate parameters from the native
range of Chinese tallow to conditions in North America. This model predicted that
Chinese tallow is capable of expanding 500 km north and well inland of its current U.S.
distribution. In addition, field experiments on germination of seeds planted beyond
Chinese tallow’s current range matched the climate-based range estimates produced by
CLIMEX (Pattison and Mack 2008), and indicated that the current range limits are
largely the result of dispersal limitation (Pattison and Mack 2009). However, the field
experiments used to test the predictions of the CLIMEX model only included seeds
collected from one coastal South Carolina population, which may or may not represent
the population with the most potential to spread inland.

To date, most estimates of the potential range of Chinese tallow treat all
populations of Chinese tallow as having equivalent potential for range expansion (Draper
1982; Pattison and Mack 2008). However, genetically determined phenotypic differences
have been documented across different populations of Chinese tallow within the U.S.
(Cameron et al. 2000; Siemann and Rogers 2001), and genetic differences are found at
selectively neutral markers among southeastern U.S. populations (DeWalt et al.
unpublished data). Chinese tallow also suffers greater herbivory in areas of the
southeastern U.S. where it has been present longer than in areas where it was more
recently introduced, which may have contributed to genetic divergence in introduced
populations in allocation to defense vs. growth and reproduction (Siemann et al. 2006).
Native ecotypes of Chinese tallow have been shown to produce more rapid growth, larger
seeds, and more poorly defended tissue (Siemann and Rogers 2001). U.S. populations of
Chinese tallow have also been found to be more tolerant of herbivory than native
populations, showing increased damage due to herbivory (Zou et al. 2008), as well as increased compensatory growth after damage (Rogers and Siemann 2004). Thus, the ability of different genotypes to recruit successfully beyond Chinese tallow’s documented invasive range may vary.

The native source population(s) of introduced genotypes in the United States is not known, but there is evidence that coastal South Carolina and Georgia populations are genetically distinct from other populations in the southeastern United States possibly because they originated from different source populations or combinations of source populations (DeWalt et al. unpublished data). The potential for these genotypes to spread northward or inland may differ, particularly if Chinese tallow from different source populations are dissimilar in their cold hardiness and rapidity of budbreak after dormancy.

This study examines whether climatic factors prevent Chinese tallow from expanding inland of its current coastal distribution in South Carolina, and whether there are genetically determined differences among Chinese tallow populations that affect germination success or seedling survival in different environments and cold regimes. We used seeds collected from North and South Carolina to examine differences among invasive populations, as well as seed from areas in the northern and southern portions of its range in China (called north and south China, respectively). We asked the following questions: 1) do populations of Chinese tallow vary in their germination success across the three major edaphic and climatic regions of South Carolina (coastal, midlands, and piedmont), 2) are differences in germination across these regions driven by winter
conditions within each region, and 3) do seedlings differ in the extent of cold induced
stem damage, budbreak timing, or overwinter survival rate under different winter regimes
or freeze durations? Climatic conditions in the northern and southern portions of Chinese
tallow’s native range differ much more widely (Table 1) than the conditions in its current
range in the Carolinas; therefore, differences in range limitation might be expected
between north and south China seeds or seedlings but not between North and South
Carolina seeds. To address these questions, we conducted two field experiments and two
lab experiments.
CHAPTER TWO

METHODS

Field germination

For the purposes of this experiment, South Carolina was divided into three regions that correspond to major transitions in soil type and minimum daily temperatures: piedmont, midlands, and coastal regions. Within each region, three sites were established in hardwood forest and placed near water sources in comparably moist conditions (Fig. 1). One piedmont field site was flooded during the course of the experiment, and therefore this field site was omitted from all analyses.

Seeds collected from trees in South Carolina, North Carolina, northern China, and southern China were used in this experiment. Henceforth, we refer to these areas as source populations, although they may encompass several populations within each area. Seeds were collected in South Carolina from 18 trees in the Ace Basin National Wildlife Refuge, the cities of Monk’s Corner and Mt. Pleasant, and the Hobcaw Barony Wildlife Refuge in November 2005. All collection sites were located within 30 miles of the coastline. Seeds were collected in North Carolina at the same time from 17 trees in the city of Wilmington. North Carolina source populations were located at 34.2° N, and South Carolina source populations ranged from 32.5° to 33.4° N (Table 1). The China seeds used in this experiment were collected in 2003 and consisted of seeds collected from 14 trees from the provinces of Anhui, Hubei, Zhejiang, and Jiangsu, which were considered to be northern China populations (henceforth called north China), as well 16
trees from Guangdong, Guangxi, Hunan, and Jiangxi provinces, which were considered to be southern China (south China). North China sites ranged in latitude from 30.2° to 32° N latitude; south China sites were located from 23.1° to 26.6° N. Average temperature of the coldest month and annual rainfall data was based on data prepared by Leemans and Cramer (1991). Conditions at collection sites in north China are substantially colder than those found at the most extreme edges of Chinese tallow’s documented U.S. range (Table 1). The average temperature of the coldest month among North China collection sites was 5°C, and 9°C in North Carolina collection sites. The average temperature of the coldest month in north China was also below the average temperature of any planting location used in this experiment (6.5°C at coldest planting location). Seeds collected from China were stored at 5°C until 2005, at which point they were stored in an air-conditioned room. Seeds collected from the Carolinas were stored in an air-conditioned room until planting.

The waxy sarcotesta surrounding all seeds was removed by stirring seeds in a water and soap mixture (Alconox, White Plains NY). The removal of the sarcotesta of Chinese tallow seeds has been found to reduce the germination time and result in a higher germination during the first year (E. Siemann and W. E. Rogers, unpublished data). In addition, it simulates passage through a bird’s digestive system, which removes most of the sarcotesta (Renne et al. 2001). This mechanism may function by increasing the rate at which the seeds imbibe water, which may aid in stimulating germination (Conway et al. 2000). In addition, removal of the sarcotesta removed any surface fungi or bacteria.
In late November 2006, 43 pieces of 15-cm diameter PVC pipe were placed in a 1-m interval grid at each site. These pipes were buried so that 2.5 to 3.5 cm remained above ground, and soil was placed in the pipes at the same level as the soil surrounding the pipe. In each pipe, five seeds from one source population were buried 1.5 cm below the soil surface. This depth of planting reduced seed movement within pipes. Planting at this depth has been shown to be within the optimal range of planting depth to stimulate germination of Chinese tallow (Donahue et al. 2004; Renne et al. 2001). Source populations were assigned to pipes in a completely randomized design for each site with a total of 10 replicate pipes for each source population. Hardware cloth was fixed over the mouth of all pipes to prevent rodents, birds, or other animals from removing seeds. Germination success was recorded in October 2007. Previous experience showed that germinated seeds could be detected even if the resulting seedling did not survive.

In three pipes at each site, Hobo pendant temperature sensors (Onset Computer Corp., Pocasset, MA) were buried at the same depth as the seeds and set to record temperature hourly for the duration of the experiment. To determine the extent to which seeds were insulated by the pipes, a sensor was buried adjacent to one pipe containing a sensor at one site from each region (Clemson, Sandhills, and Coastal R.E.C.). No significant differences were detected between temperature sensors placed within and outside of pipes ($F_{1,461}=0.00 P=0.98$).

To determine whether germination was driven by winter temperatures as opposed to edaphic or biotic differences between regions, additional pipes with a similar but independently randomized design were placed adjacent to each existing site in mid-
March 2007, after the frost date. Germination rates of overwintered seeds (those placed in the ground in November 2006) were then compared with non-overwintered seeds (those placed in the ground in March 2007). These are referred to as fall and spring plantings, respectively. All resulting seedlings were recorded and removed throughout the year. Second-year germination was also recorded in August 2008, at which point all remaining material was removed from each site.

All statistical analyses were conducted using SAS/Stat v9.1. The effects of region, overwintering, and source population on seed germination were examined using Proc Glimmix. Because each seed either germinated or failed to germinate, the data were analyzed using a binomial response distribution. To reduce the Type 1 error rate, the p-values for all effects were adjusted according to Sidak’s inequality. Because viability of Chinese tallow seeds kept in dry storage declines 2-3 years after collection (Bruce et al. 1997; Cameron et al. 2000), direct comparisons of the germination of seeds from Carolina populations (collected in 2005) and China populations (collected in 2003) were not appropriate. Therefore, all comparisons of germination were conducted only between Carolina or between China populations (using Proc Glimmix).
Fig. 1 Locations of substantial naturalized populations of Chinese tallow in the United States are shown in gray shading, and field sites used in South Carolina are shown in the inset. Seeds were planted at each site in two adjacent grids in November 2006 and March 2007. Site numbers correspond to those in Table 1. Lines within South Carolina denote approximate changes in region according to major transitions in soil type. The limit of the current invasive range of Chinese tallow roughly corresponds to the soil transition between the coastal and midlands. Large naturalized populations of Chinese tallow currently occur only in the coastal region, although scattered trees can be found throughout the state.
Table 1 Mean temperature and annual rainfall of seed collection sites and field sites used to test germination success of Chinese tallow tree in three regions of South Carolina. Meteorological data were obtained from a global weather dataset prepared by Leemans and Cramer (1991). Data were not available (NA) for Guizhou and Guangxi provinces.

<table>
<thead>
<tr>
<th>Collection Sites</th>
<th>Province/Site</th>
<th>Average Temperature of Coldest Month (°C)</th>
<th>Annual Rainfall (mm)</th>
<th>Latitude (N)</th>
<th>Longitude (W)</th>
<th>Min. Soil Temp. °C</th>
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<tr>
<td>North China</td>
<td>Anhui</td>
<td>3</td>
<td>1050</td>
<td>31.59°</td>
<td>117.18°</td>
<td>-</td>
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<tr>
<td></td>
<td>Zhejiang</td>
<td>5</td>
<td>1200</td>
<td>30.288°</td>
<td>120.14°</td>
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<tr>
<td></td>
<td>Jiangsu</td>
<td>1.5</td>
<td>950</td>
<td>32.037°</td>
<td>118.84°</td>
<td>-</td>
</tr>
<tr>
<td>South China</td>
<td>Fujian</td>
<td>12.5</td>
<td>1150</td>
<td>24.45°</td>
<td>118.09°</td>
<td>-</td>
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<tr>
<td></td>
<td>Jiangxi</td>
<td>8</td>
<td>1450</td>
<td>26.65°</td>
<td>114.65°</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Hunan</td>
<td>7.5</td>
<td>1250</td>
<td>29.8°</td>
<td>111.3°</td>
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<tr>
<td></td>
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<td>NA</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td></td>
<td>Guangdong</td>
<td>14</td>
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<td>NA</td>
<td>-</td>
<td>-</td>
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<tr>
<td>North Carolina</td>
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<td>9</td>
<td>1300</td>
<td>34.2°</td>
<td>-77.9°</td>
<td>-</td>
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<tr>
<td></td>
<td>USS NC</td>
<td>9</td>
<td>1300</td>
<td>34.2°</td>
<td>-77.9°</td>
<td>-</td>
</tr>
<tr>
<td>South Carolina</td>
<td>Hobcaw</td>
<td>9.5</td>
<td>1150</td>
<td>33.38°</td>
<td>-79.9°</td>
<td>-</td>
</tr>
<tr>
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<td>Moncks Corner</td>
<td>9</td>
<td>1200</td>
<td>33.19°</td>
<td>-80.01°</td>
<td>-</td>
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<tr>
<td></td>
<td>Ace Basin</td>
<td>10.5</td>
<td>1100</td>
<td>32.52°</td>
<td>-80.3°</td>
<td>-</td>
</tr>
</tbody>
</table>

Field Sites

| Piedmont         | Clemson      | 6.5                                      | 1100                 | 34.62°       | 82.8°         | 1.14              |
|                  | Greenwood    | 7.5                                      | 1200                 | 34.11°       | 82.12°        | 2.59              |
| Midlands         | PeeDee       | 7.5                                      | 1150                 | 79.3°        | 34.3°         | 3.64              |
|                  | Sandhills    | 7.5                                      | 1150                 | 80.87°       | 34.13°        | 4.13              |
|                  | Edisto       | 8.5                                      | 1100                 | 81.32°       | 33.35°        | 2.87              |
| Coastal          | Hobcaw       | 9.5                                      | 1150                 | 79.16°       | 33.2°         | 5.64              |
|                  | Coastal      | 10.5                                     | 1100                 | 80.4°        | 32.47°        | 5.38              |
|                  | Nemours      | 10                                       | 1150                 | 80.73°       | 32.6°         | 3.65              |
**Seedling field response to winter conditions in the piedmont**

As determined by Renne (2001) overwinter mortality in Chinese tallow occurs primarily in plants under 30 cm tall, suggesting that the seedling stage is critical in limiting expansion into areas with colder winter conditions than coastal areas. To determine the effects of winter conditions in the piedmont on the growth and survival of Chinese tallow, seedlings from the four source populations were planted in a forested site in the piedmont region of South Carolina in October 2007 in the South Carolina State Botanical Garden. Seedlings were arranged in a 1-m spaced grid according to a randomized block design. Twenty North Carolina seedlings, 20 South Carolina seedlings, 15 north China seedlings, and 10 south China seedlings raised in a growth chamber were used. In addition to the planted seedlings, this site also contained five deliberately planted Chinese tallow trees and 11 naturalized seedlings (presumably from these adult trees) that were located immediately surrounding the planted seedlings. Survival, stem damage, and bud status of these seedlings were monitored from November 2007 to June 2008. Stem damage was monitored through repeated measurement of living stem tissue height throughout the winter. When possible, measurements were taken immediately before and immediately after incidence of freezing temperatures. Height of all seedlings was recorded before the first frost, which occurred on 18 December 2008. Once seedlings first began to break bud in mid-April, the height of all seedlings was measured and compared with the heights recorded in the fall. Measurements of survival and bud status continued through June 18, 2008.
Once initial budbreak was recorded among seedlings, budbreak was recorded among all seedlings on a weekly basis. Mean vector and repeated measures analyses were conducted using Proc Mixed on stem height collected throughout the fall and winter to test whether there were differences in cold hardiness between seedlings from different source populations. Differences among source populations in relative proportion of seedling height loss (\((\text{initial height} - \text{final height})/\text{initial height}\)) after freezing temperatures were examined using Proc Glimmix using a normal probability distribution.
Seedling lab response to timing of freeze events

This experiment tested the survival, growth, and time to budbreak of Chinese tallow seedlings with and without exposure to a freeze event during dormancy, as well as the proportion of seedlings that experienced immediate damage caused by freeze events before and after budbreak. Thirty-eight North Carolina, 34 South Carolina, 35 north China, and 21 south China seedlings were kept in a cold chamber at 5°C for 4 mo to fulfill cold requirements. At the end of this period, all seedlings had entered a dormant state, and measurements of height, bud damage, and stem damage were recorded. To test the effects of freezing conditions on dormant and active seedlings, all seedlings were split into two groups, and approximately half (19 North Carolina, 16 South Carolina, 18 north China, and 10 south China) were placed in a freezer and exposed to freezing conditions. These seedlings are referred to henceforth as the ‘dormant, freeze-treated’ seedlings. The temperature was reduced from 5°C to -5°C at a rate of 1°C/hour and maintained at -5°C for six hours. The temperature was then increased to 5°C at the same rate. Seedlings were maintained at 5°C for 24 hours, at which time all measurements were repeated and damage resulting from exposure to -5°C was recorded. All seedlings that were not exposed to a freezing treatment were also measured, and all seedlings from both treatments were placed in a 25°C chamber. Seedlings from both treatments were monitored daily for budbreak and type of initial bud (lateral or apical) was recorded. After budbreak, seedlings that were not exposed to a freeze event during dormancy (henceforth referred to as ‘post-dormancy, freeze-treated’ seedlings) were placed into a 5°C chamber for 24 hours, and then exposed to freezing temperatures in the same manner.
as the dormant, freeze-treated seedlings. After the seedlings were returned to the 25°C chamber for 24 hours, stem and leaf damage was recorded to determine the vulnerability of recently budding seedlings to damage from frost events that occurred after budbreak. Seedlings were monitored for 70 days after initial placement in the 25°C chamber.

Statistical analyses were conducted using Proc Glimmix on the proportion of seedlings damaged by -5°C conditions, number of seedlings that broke bud, seedling mortality by the end of the experiment, and number of days at 25°C before budbreak to determine the relative effect of freezing conditions both before and after budbreak on the different source populations of Chinese tallow. An analysis was also conducted on the relative number of seedlings that displayed apical as opposed to lateral buds because regrowth from lateral buds might reflect damage to apical buds that was not obvious on dormant seedlings.
Seedling lab response to extended freeze durations

Survival, growth, and damage to all four populations of Chinese tallow seedlings after exposure to different durations of freezing temperatures was also determined. A group of 56 North Carolina, 34 South Carolina, 15 north China, and 26 south China seedlings were germinated and grown at 25°C under a 12-hour light cycle, and then exposed to different freeze durations. Before exposure to freezing temperatures, all seedlings were exposed to five days of alternating temperatures of 25°C when under light and 5°C under dark conditions. Height was measured for all seedlings prior to exposure to freezing conditions. Seedlings were then placed in a 5°C chamber for 24 hours, and the temperature dropped to -7°C at a rate of 1° per hour. Seedlings were maintained at -7°C for 6, 48, 96, 192, or 384 hours. After treatment, temperature was returned to 5°C in the same fashion, and seedlings were maintained at 5°C for 24 hours. Height, extent of stem damage, and presence of bud damage were recorded, and seedlings were returned to 25°C. After 6 weeks, seedling height and survival were recorded again and changes in height were contrasted across treatments and source populations. Regression and logistic regression analyses were conducted using Proc GLM and Proc Glimmix, respectively.

Although it would be more realistic to model progressively colder temperature minima and hold the duration of the treatment more constant, this method was used due to the minimal response of seedlings to short freezes at the temperatures that were attainable using the available equipment.
Significant differences were determined at the $P \leq 0.05$ level. We refer to $0.1 \leq P < 0.05$ as marginally significant if other experimental results suggested that the difference among treatments could be biologically meaningful.
CHAPTER THREE
RESULTS

Field germination

Source populations differed significantly in germination success (Table 2), as North Carolina seeds had higher germination than other source populations included in this study (Fig. 2). However, germination success of different source populations depended on planting season and region of planting (i.e. there was a significant source population*planting season*region of planting interaction, $F_{6,588}=2.64, P=0.016$). In fall but not in spring plantings, germination of South Carolina seeds was marginally significantly lower in piedmont than coastal sites (Fig. 2). Germination of North Carolina seeds was lower in midlands sites than coastal or piedmont sites regardless of planting season. North Carolina seeds had higher germination success than South Carolina seeds in fall-planted piedmont sites (Fig. 2); however, no differences in germination were detected between North Carolina and South Carolina seeds in spring plantings. No significant differences in germination of north China and south China seeds were detected across planting season or region.

Germination occurred primarily in the first year of planting. No second year germination occurred in four sites, and second-year germination of more than one seed was detected in only two sites. Inclusion of second year germination data did not alter the significance or interpretation of the results, and was therefore excluded from these analyses.
Table 2 Type 3 test of fixed effects for germination of different genotypes of Chinese tallow seeds in different regions of South Carolina. Significant p-values are in boldface. Although no significant differences in germination were detected among sites within a region, the inclusion of between-site random effects into Proc Glimmix resulted in non-integer degrees of freedom for some parts of the analysis.

<table>
<thead>
<tr>
<th>Effect</th>
<th>df</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Source Population (P)</td>
<td>3, 588</td>
<td>12.99</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Planting Region (R)</td>
<td>2, 10.09</td>
<td>1.21</td>
<td>0.337</td>
</tr>
<tr>
<td>Planting Season (S)</td>
<td>1, 10.09</td>
<td>0.48</td>
<td>0.503</td>
</tr>
<tr>
<td>G*R</td>
<td>6, 588</td>
<td>4.79</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>G*S</td>
<td>3, 588</td>
<td>4.62</td>
<td>0.003</td>
</tr>
<tr>
<td>R*S</td>
<td>2, 10.09</td>
<td>0.39</td>
<td>0.687</td>
</tr>
<tr>
<td>G<em>R</em>S</td>
<td>6, 588</td>
<td>2.64</td>
<td>0.016</td>
</tr>
</tbody>
</table>
Fig. 2 Germination of Carolina and China seeds by region of planting for fall (a,b) and spring plantings (c, d). Different lowercase letters denote significant differences between Carolina (a, c) or China (b, d) source populations among planting regions for each planting season as determined by LSmeans comparisons. Black bars represent South Carolina seeds, light gray bars represent North Carolina seeds, dark gray bars represent south China seeds, and white bars represent north China seeds. In both fall and spring plantings, germination success of North Carolina seeds was significantly greater than South Carolina seeds in coastal and piedmont sites, but not in the midlands. North China and south China seeds did not differ in germination success in any of the regions in either planting season.
Seedling field response to winter conditions in the piedmont

Relative height loss of field-planted seedlings differed among source populations for some dates of measurement, but not others (i.e. significant source population*date of measurement interaction; $F_{30,570}=1.48, P=0.05$; Fig. 3). During the periods when differences between source populations were most pronounced, North Carolina seedlings showed less height loss than South Carolina seedlings, north China less than south China, and north China less than South Carolina. These differences in height loss were greatest from mid-January to March, as increasing damage to all seedlings later in the season reduced the dissimilarities among source populations. However, North Carolina seedlings showed significantly less loss of height than South Carolina seedlings through the end of the experiment (Fig. 3).

Overall seedling mortality was 60.3%, and there were no significant differences among source populations in survival ($F_{3,61}=0.80, P=0.50$) or date of budbreak ($F_{3,34}=0.57, P=0.64$). South Carolina seedlings tended to be less likely to successfully break bud after enduring piedmont winter conditions than other source populations ($F_{3,61}=2.3, P=0.09$).
Fig. 3 Relative height loss of seedlings planted in the South Carolina State Botanical Garden, Clemson, SC. Significant differences in height loss were detected across dates between North Carolina and South Carolina source populations ($F_{1,60}=2.36$, $P=0.02$). Asterisks denote dates for which significant differences were detected. Measurements began before the first frost, which was two weeks after initial planting, and continued until budbreak began in late April, well after the last frost.
Seedling response to freeze timing under lab conditions

Freeze-treated, dormant seedlings had significantly greater survival rates than seedlings that were not frozen until after budbreak (Fig. 4), but source population did not affect survival of freeze-treated seedlings (Table 3). In addition, fewer dormant, freeze-treated seedlings broke dormancy from apical buds than post-dormancy, freeze-treated seedlings. No effects of source population or timing of freeze events were detected on post-freeze stem damage, length of time between removal from 5°C and budbreak (Table 3), or number of seedlings that broke bud (Table 3).

Table 3 Type 3 test of the effects of genotype (numerator df = 3), freeze timing (ndf = 1), and their interaction (ndf=3) on survival (denominator df=121), bud type (ddf=66), and budbreak success (ddf=66) of Chinese tallow seedlings under lab conditions. Significant p-values are in boldface.

<table>
<thead>
<tr>
<th>Effect</th>
<th>Survival</th>
<th>Budbreak</th>
<th>Stem Damage</th>
<th>Time to Budbreak</th>
<th>Effect on Bud Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype (G)</td>
<td>F</td>
<td>P</td>
<td>F</td>
<td>P</td>
<td>F</td>
</tr>
<tr>
<td>Freeze Timing (F)</td>
<td>8.05</td>
<td><strong>0.005</strong></td>
<td>1.29</td>
<td>0.259</td>
<td>1.01</td>
</tr>
<tr>
<td>G*F</td>
<td>2.28</td>
<td><strong>0.083</strong></td>
<td>1.06</td>
<td>0.368</td>
<td>0.61</td>
</tr>
</tbody>
</table>
Fig. 4 Survival of dormant, freeze-treated and post-dormant, freeze-treated seedlings six weeks after exposure to 5°C. Although there was an effect of freeze timing on seedling survival, no significant effects of freeze treatment were detected for seedlings of a single genotype. Dark grey bars represent seedlings exposed to freezing conditions while dormant, and light grey bars represent post-dormant frozen seedlings. Letters indicate significantly differences between treatments and genotypes.
Seedlings response to extended freeze durations

Main effects of source population \((F_{3,117}=2.97, P=0.04)\) and freeze duration \((F_{1,117}=19.51, P<0.001)\) but not their interaction \((F_{3,114}=0.65, P=0.58)\) were detected on seedling survival (Fig. 5). Estimates of survival pooled across freeze durations showed that North Carolina seedlings were significantly more likely to survive freezes than South Carolina seedlings, and that south China seedlings had significantly higher survival rates than South Carolina seedlings (Fig. 5). Height loss, as measured both immediately after freezing as well as six weeks after freezing, did not significantly differ among source populations.

![Bar chart showing percent survival after six weeks for different genotypes.](image)

**Fig. 5** Average survival rate of Chinese tallow seedlings six weeks after exposure to 6, 48, 96, 192, or 384 hours at -7°C. Letters indicate significant differences between genotypes.
CHAPTER FOUR

DISCUSSION

Interpretation

Although climate may be the primary determinant of plant species’ range, it is clear that other factors also play a role in determining the range of a species. In the case of Chinese tallow, differing physiological tolerances to cold appear to exist between populations within its invasive range, which may result in different range limitations. As other studies have also found evidence that there are phenotypic differences between Chinese tallow from native and invasive populations as well as among invasive populations (Rogers and Siemann 2002; Rogers and Siemann 2004; Siemann and Rogers 2001; Siemann and Rogers 2003; Zou et al. 2007; Zou et al. 2008), it is probable that at least some populations of Chinese tallow have different physiological responses to climate that result in different limits to their potential range. Although post-invasion genetic shifts similar to those found in Chinese tallow have been found in a number of other invasive plant species (Bossdorf et al. 2005; Cano et al. 2008; DeWalt et al. 2004; Meyer and Hull-Sanders 2008), there has been little research examining physiological differences of an invasive species within its invasive range. Thus an examination of the physiological tolerances of populations with Chinese tallow’s invasive U.S. range may provide insight concerning a largely overlooked but potentially important factor in the determination of the potential range of invasive plant species.
Of the four source populations examined in this study, the South Carolina genotypes appear to have the least potential for invasive expansion. Seeds from the South Carolina source population showed lower germination success in the piedmont (12.5%) than the coastal region (28.6%). Winter conditions appear to drive the lower germination in inland sites because lower germination was only found for fall-planted and not spring-planted seeds in piedmont and midland sites. South Carolina seedlings also displayed greater overwinter stem damage in the field, slightly lower budbreak success in the spring, and lower survival after freezing events than North Carolina or China seedlings. In accordance with our results, Cameron (2008) found that South Carolina seeds planted in a glasshouse under natural temperatures on the Texas coast germinated more slowly than seeds from Florida, Taiwan, Texas, or Georgia, and the resulting seedlings had lower survival rates. Thus, the genotypes of Chinese tallow currently in South Carolina likely pose less risk of inland, and possibly northward, expansion than those in other southeastern U.S. populations.

Germination of North Carolina seeds was significantly reduced in the midlands as compared to coastal and piedmont sites. As this pattern persisted across both fall- and spring-planted seeds, winter conditions do not account for this effect. Anecdotally, observations of wilting among seedlings suggested that drought stress was severe in midlands sites, which may account for this reduction in germination. Soils at midlands sites had a higher sand content than the piedmont, and were more prone to desiccation than coastal sites. Bruce (1993) found that the survival of Chinese tallow seedlings exposed to drought conditions was reduced in soils with high sand content as opposed to
clay soils. Because of the greater water-holding capacity of clay than sandy soil, higher rainfall may be required for sufficient water imbibition by seeds to stimulate germination in sites with high sand content in the soil (Bruce 1993; Conway et al. 2000). Although germination of North Carolina seeds was reduced in midlands sites, the rate of germination was similar to that of other source populations across all regions, including coastal sites where Chinese tallow is currently abundant. Thus, the reduction in germination of North Carolina seeds in the midlands does not indicate an exclusion of the North Carolina population from the midlands of South Carolina.

A number of non-genetic factors may also lead to differing germination requirements between spatially distinct populations, such as dissimilar predator populations or differences in soil biota, as well as the environmental history of the parent plants. In some cases, environmental conditions of maternal plants has led to differences in characteristics such as seed size or permeability of the seed coat, which may affect the germination requirements of seeds (Baskin and Baskin 1973; Quinn 1977). Thus, unless plants are grown under common garden conditions for one or more generations in order to remove the effect of maternal climate history, differences in the climate conditions of plant populations may affect the physiological response of their seeds and seedlings even if no genetic separation exists between populations (Nelson et al. 1970).

North and south China seeds had similar germination rates, and their seedlings responded similarly to freezes. As the China source populations displayed the greatest difference in historic climate conditions, it does not appear that the maternal environment substantially affects germination or seedling response to freezing in Chinese tallow.
Because North and South Carolina populations of Chinese tallow exist in areas where winter conditions are relatively similar, we infer that differences in germination and overwinter seedling damage between Carolina populations are likely genetically determined. Different native provenances or divergence since introduction could account for these genetic differences. The Carolina populations also differ in allele composition at selectively neutral loci, suggesting that different native provenances or mixtures of native source populations have contributed to differences in performance (DeWalt et al. unpublished data).

Reductions in germination of fall-planted seeds among the South Carolina source population in non-coastal regions may be due not only to cold conditions but also to longer exposure to soil pathogens and lower resistance of South Carolina populations than other sources to soil pathogens. Fungal parasites that attack seeds of Chinese tallow and render them nonviable have been detected in its U.S. range (Draper 1982; Scheld et al. 1980). In addition, Chinese tallow has been found to exhibit significant feedback inhibition in soil where it is established in the U.S. (Nijjer et al. 2007b). However, beneficial effects of soil biota on survival and growth have been found in areas where it has not been historically present (Nijjer et al. 2007a). Therefore, it is unlikely that exposure to soil pathogens caused a reduction in Chinese tallow germination in the piedmont, which was far from established stands and thus likely contained a naïve soil biota.
Mechanisms and implications for possible range expansion

These experiments show that prediction of the range of invasive species must take into account the potential for different populations of an invasive species to have different physiological tolerances and thus different potential ranges. At least some populations of Chinese tallow have different physiological responses to climate that likely result in different limits to their potential range, and that physiological differences between populations within an invasive range may play a significant role in the expansion and potential range of that species.

The series of experiments reported here also demonstrate that the potential range of Chinese tallow tree is likely much broader than the current distribution along the coast in the southeastern U.S. Seeds of Chinese tallow tree appear capable of germinating successfully throughout the coastal, midlands, and piedmont regions of South Carolina. These results are consistent with the range predictions of Climex models (Pattison and Mack 2008). Some source populations differed in their potential for invasion of inland habitats, but germination levels were probably not low enough to preclude expansion of Chinese tallow once an initial population is established, given the intense seed rain that has been found within established populations elsewhere (Siemann and Rogers 2006).

Because seeds collected in South Carolina showed low germination due to winter conditions in non-coastal regions while seeds collected in China did not, some loss of tolerance to cold conditions as Chinese tallow naturalized along coastal South Carolina may have occurred. However, no differences in survival or cold hardiness were detected between North Carolina and China seedlings, indicating that little to no loss in cold conditions...
hardiness has occurred in the North Carolina population of Chinese tallow. This result would contradict the hypothesis of post-invasion selection for lower cold hardiness. However, as North Carolina represents the northernmost edge of Chinese tallow’s current U.S. range, it may be that the selective pressures on that population differ significantly from more southern populations.

Field experiments conducted by Pattison and Mack (2008; 2009), using seeds collected from South Carolina, indicated that Chinese tallow is capable of germinating far beyond its current range in the U. S. and up to 500 km north of its current range, although germination was lower among more inland and northerly sites. Our experiments indicate, however, that genotypes of Chinese tallow in South Carolina may be more limited by cold than North Carolina populations, as similar reductions in germination were only detected among seeds collected from South Carolina. Thus, the potential invasive range of Chinese tallow tree in the U.S. may be even larger than previously estimated.

In addition, while conducting this research, we found a naturalized stand of Chinese tallow in a recently cleared forest area in Clemson SC, indicating that successful recruitment can occur in the piedmont. Winter conditions in the piedmont likely act to reduce the likelihood of initial establishment, but may not be sufficient to prevent expansion of an established population that acts as a continual seed source. As Chinese tallow genotypes currently established in North Carolina have the greatest germination success and seedling survival in inland areas, management efforts should prioritize control of the North Carolina population to prevent the spread of Chinese tallow.
Other mechanisms, such as limited seed dispersal inland, may also inhibit Chinese tallow establishment in midlands and piedmont areas. Chinese tallow seeds are dispersed by water and by birds (Bruce et al. 1997). As most river systems in South Carolina flow towards the coast, dispersal by water may not result in substantial inland movement of seeds. In addition, most bird species that feed on seeds of Chinese tallow in South Carolina are winter residents that do not move substantially inland or northward when mature seeds are available, and the majority of seeds are consumed well before spring migration northward begins (Renne et al. 2002). Thus, neither of the predominant, natural dispersal mechanisms of Chinese tallow would spread seeds inland. Given the extensive use of Chinese tallow as an ornamental plant and the use of seeds in wreaths and other ornaments sold to tourists along the coast, however, it seems implausible that dispersal of seeds into the midlands and piedmont of South Carolina can be prevented indefinitely. With increasing interest in the cultivation of Chinese tallow in the Americas for fiberboard, lumber, and biodiesel production (Lee et al. 2004; Shupe et al. 2008; Shupe et al. 2006), it is likely that inland dispersal of Chinese tallow in the future will be due at least partly to anthropogenic activities.

Post-invasion genetic shifts in growth rate (Cano et al. 2008; DeWalt et al. 2004), resource allocation (Meyer and Hull-Sanders 2008), and defense (Bossdorf et al. 2005; Siemann and Rogers 2001) have been detected in a variety of invasive plant species. In addition, Donahue (2005b; 2005c) showed that differences in the rate of evolution post invasion may control both the rate of range expansion by an invader as well as affecting the life history strategy developed by the resulting population. Differences in
physiological tolerances across invasive populations, such as are found in Chinese tallow, may exist within many invasive species. Thus, in studying post invasion selection and determining the potential invasive range of a species, it is necessary to consider not only the physiological differences between native and invasive populations of plant species, but also possibility of physiological differences between invasive populations that could lead to different range limits.
Chinese tallow is an invader to the southeastern United States whose potential range is largely unknown. However, there is evidence that the potential for range expansion is not identical across Chinese tallow populations in the Carolinas. Numerous studies have been conducted on the physiological differences between native and U.S. populations of Chinese tallow, but many studies to date treat all U.S. populations of Chinese tallow as a single unit. This is problematic, as it may lead to overly conservative predictions of its potential spread. This study shows the importance of considering differences between invasive populations as well as differences between populations from the invasive and native range in understanding physiological responses to climatic and biotic stress factors and predicting range limits of invasive species. Management plans based on characteristics of plants from one population may not hold true for plants drawn from another population.
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