

Differences in seed properties and germination between native and introduced populations of *Triadica sebifera*

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Abstract

Aims Seeds of many invasive plants germinate more quickly than those of native species, likely facilitating invasion success. Assessing the germination traits and seed properties simultaneously for introduced and native populations of an invasive plant is essential to understanding biological invasions. Here, we used *Triadica sebifera* as a study organism to examine intraspecific differences in seed germination together with seed characteristics.

Methods We measured physical (volume, mass, coat hardness and coat thickness of seeds) and chemical (crude fat, soluble protein, sugar, gibberellins [GA] and abscisic acid [ABA] of kernels) properties of *T. sebifera* seeds collected in 2017 from 12 introduced (United States) populations and 12 native (China) populations and tested their germination rates and timing in a greenhouse experiment in China. Furthermore, we conducted an extra experiment in the United States using seeds collected in 2016 and 2017 to compare the effects of study sites (China vs. United States) and seed collection time (2016 vs. 2017) on seed germination.

Important Findings Seeds from the introduced range germinated faster than those from the native range. Physical and chemical measurements showed that seeds from the introduced range were larger, had higher GA concentrations and GA:ABA ratio, but lower crude fat concentrations compared to those from the native range. There were no significant differences in seed mass, coat hardness and coat thickness or kernel ABA, soluble protein or sugar concentrations between seeds from introduced vs. native ranges. Germination rates were correlated between United States and China greenhouses but germination rates for populations varied between collection years. Our results suggest that larger seeds and higher GA likely contribute to faster germination, potentially facilitating *T. sebifera* invasion in the introduced range.

Keywords: invasive plant, germination, seed volume, gibberellins, plant invasion

摘要: 很多入侵植物的种子比原产地植物的种子萌发的更快, 这可能有利于它们成功入侵。为了解生物入侵过程, 对入侵植物的入侵种群和原产地种群的种子特性和萌发特征同时进行评估极为必要。本研究中, 我们以乌桕(*Triadica sebifera*)为研究对象探究种子萌发和种子特征的种内差异。我们利用采于2017年的乌桕种子, 来自中国的12个原产地种群和美国的12个引入地种群, 测定种子的物理性状(体积、重量、种皮硬度和种皮厚度)和化学特性[种仁的粗脂肪、可溶性蛋白质、糖、赤霉素(GA)和脱落酸(ABA)], 并在中国的温室中测定种子的发芽率和萌发时间。此外, 我们在美国用采自2016年和2017年的种子开展了研究地点(中国和美国)和种子采集年份(2016年和2017年)对种子萌发影响的比较实验。来自引入地的种子要比来自原产地的种子萌发更快。种子的物理和化学特性测定结果表明: 与原产地种子相比, 引入地种子更大、种仁内含有的赤霉素浓度以及GA:ABA比更高, 但粗脂肪含量较低。种子重量、种皮硬度、种皮厚度、种仁脱落酸、可溶性蛋白质和

糖含量在引入地和原产地之间没有显著差异。在中国和美国温室间的种子萌发率具有相关性，但种群的萌发率具有年际变化。我们的研究结果表明，体积较大和赤霉素含量较高的种子可能有助于快速萌发，这可能也有利于乌桕在引入地的入侵。

关键词：入侵植物、种子萌发、种子体积、赤霉素、植物入侵

INTRODUCTION

Seed germination is a critical stage in the lifecycle of higher plants, because this transition impacts plant fitness, population dynamics and ultimately influences their evolutionary direction (Chen *et al.* 2012; Muscarella *et al.* 2013). Successful germination of invasive plants can play an important role in their establishment and expansion in the introduced range, and thus in their invasion potential (Ferrerias and Galetto 2010; Gioria and Pyšek 2017). The factors influencing seed germination are often attributed to the properties of the seeds themselves, including seed size (Dunlap and Barnett 1983), seed coat hardness or permeability (Rolston 1978), and seed reserves and hormones (Kucera *et al.* 2005; Lv *et al.* 2018). Thus, examining the seed properties related to germination of invasive plants could give a better understanding of the importance of these seed traits in plant invasions.

Comparative studies of closely related invasive and non-invasive species in their introduced ranges can reveal trait differences that contribute to invasiveness of an introduced species within invaded environments (Gioria and Pyšek 2017). Several studies have shown that invasive plants may germinate earlier and/or more rapidly under a broader array of conditions than their non-invasive congeners (Erfmeier and Bruelheide 2005; Udo *et al.* 2017). More importantly, intraspecific studies of introduced and native populations are needed to determine the mechanisms of the invasion process and the ecological and evolutionary processes promoting the success of invasive species (Hierro *et al.* 2005). However, relatively few studies have applied this comparative method to germination traits of invasive plants (reviewed in Gioria and Pyšek 2017). Some studies have observed that seeds from introduced populations germinated better, comparable or worse under a broader range of conditions than those from native populations (Leiblein-Wild *et al.* 2014; Udo *et al.* 2017). For example, Hirsch *et al.* (2012) showed that seeds of *Ulmus pumila* from introduced populations germinated significantly faster than those from native populations. In contrast, Alba *et al.* (2016) found that native populations of *Verbascum thapsus* had a wider germination niche breadth and germinated as well as or better than introduced populations. It is not clear in these studies, however, why seeds from particular geographic ranges germinated faster or at higher rates.

Seed properties have the potential to influence seed germination (Dunlap and Barnett 1983). For example, seed physical properties including seed size, coat thickness and hardness have been found to influence the rate and speed of germination. Generally, larger seeds with thinner coats have higher germination rates and germinate faster than smaller seeds with thicker coats for a given species (Dunlap and Barnett 1983; Leverett and Jolls 2014; Suárez-Vidal *et al.* 2017). Furthermore, seed chemical properties, such as contents of proteins, lipids and carbohydrates, also play important roles in seed germination, but the relative contribution of each metabolite may depend on germination stage. One study on sugar beet use of seed reserves found that seed lipids are the main respiratory substrate, while starch and remaining lipids sustain root and hypocotyl growth (Elamrani *et al.* 1992). After initial seed imbibition, lipids and proteins are the major reserves, while sugars and starch (carbohydrates) are the minor reserves mobilized during germination

(Alencar *et al.* 2012; Suda and Giorgini 2000). Furthermore, plant hormones could ultimately determine seed dormancy. The effects of a seed's hormones on germination are relatively clear: abscisic acid (ABA) is a positive regulator of dormancy and a negative regulator of germination, while gibberellins (GA) counteract ABA effects, releasing dormancy and promoting germination (Kucera *et al.* 2005). Thus, the balance of AG and ABA is often considered an important mediator in seed germination (Shu *et al.* 2016; Tuan *et al.* 2018). To date, however, no study has linked these properties of seeds for introduced and native populations of invasive plants with their germination rates or timing.

Studies have shown that there is a positive relationship between seed germination traits (rate and speed) and plant invasion success (Colautti *et al.* 2006; Gioria and Pyšek 2017). Thus, it is reasonable to predict that seeds from introduced populations germinate faster and/or earlier and have higher values of germination reserves and promoting hormones than those of native populations. In this study, we examined the seed properties and germination of Chinese tallow tree, *Triadica sebifera* (L.) Small (Euphorbiaceae), which is native to Asia and invasive in the southeastern USA. Compared with native populations, plants from introduced populations have higher growth rates (Zou *et al.* 2007), lower resistance and higher tolerance to herbivores (Huang *et al.* 2010), and greater seed productivity (Siemann and Rogers 2001). However, whether there are differences in seed morphology, germination and physiology between introduced and native populations is still unclear. Here, we hypothesized that: (i) seeds from introduced populations will germinate faster and at a higher rate than those from native populations; (ii) seeds of introduced populations will be larger, better provisioned, and have a higher content of hormones that promote germination compared to native populations.

MATERIALS AND METHODS

Study organism

Triadica sebifera develops green fruits by late spring or early summer. Fruits are three-lobed capsules usually containing three seeds. Capsules mature in later October when capsule walls dehisce and expose globular seeds. Seeds are covered by wax which is rich in protein, lipids and fatty acids, and can persist on trees for weeks (Duke and Ayensu 1985). Seeds are dispersed mainly by birds because the wax covering is an attractive source of energy for birds (Renne *et al.* 2000).

We collected seeds from the end of October to the beginning of December in different habitats including abandoned farmlands, riversides and forest edges in both introduced (USA) and native (China) ranges. In each range, we collected seeds from 7 and 12 populations in 2016 and 2017, respectively (Supplementary Table S1). Molecular study suggests that coastal Georgia and South Carolina populations were introduced from southern China, while other US populations originate from the northeast of the native range (DeWalt *et al.* 2011). Populations used in this study are representative of the entirety of both introduced and native populations (Supplementary Table S1). For each population, we randomly selected 8–10 trees which were 10–12 m in

height and separated by at least 10 m. To simulate the germination of seeds that had passed through a bird (Renne *et al.* 2001), we soaked seeds of each population in water with detergent and scrubbed them to remove the wax. We then rinsed and stratified them in wet sand in a refrigerator at 4°C until use to simulate seeds being deposited in a suitable germination site in winter (Renne *et al.* 2001).

Seed physical and chemical properties

To examine the physical variations of *T. sebifera* seeds between introduced and native ranges, we randomly chose 10–30 seeds collected in 2017 from each of 24 populations (12 populations per range, Supplementary Table S2). We measured the length, width and height of each seed using a caliper. We treated seed shape as an oblate spheroid and calculated seed volume as length × width × height × $\Pi / 4$. Then, we weighed seeds and measured their coat hardness using an AGW Digital Grain Hardness Tester (peak mode, AGW-20, Wenzhou Yiding Aliyiqi Instrument Company, China). Finally, we randomly selected another 15 seeds from each of 21 populations, crushed them and measured the thickness of the seed coat using a caliper (12 native populations and 9 introduced populations, Supplementary Table S2).

To examine the chemical variations of *T. sebifera* seeds between introduced and native ranges, we randomly chose 90 seeds collected in 2017 from each of 24 populations (12 populations per range). We removed the coat and collected the kernel of each seed. We then randomly pooled kernels from each population in groups of 30, resulting in three biological replicates of each population (Supplementary Table S2). We immediately froze the kernels of each replicate in liquid nitrogen and ground them finely using a mortar and pestle. We used the fine powder to determine crude fat, soluble protein, sugar, GA and ABA measurements. Briefly, we measured crude fat using the Soxhlet extraction method (Anderson 2004), in which we weighed the sample (M), dried it and reweighed it (M1), then extracted the sample with diethyl ether, dried it and weighed it again (M2). We calculated crude fat concentration as $(M1 - M2) / M$. We determined soluble protein content by the Bradford method (Bradford 1976) using bovine serum albumin as the standard and Brilliant Blue G-250 as the reagent. We measured absorbance at 595 nm. We determined sugar content by the Anthrone method (Khan *et al.* 2000) using glucose as the standard and anthracene as the reagent. We measured absorbance at 620 nm. We measured GA and ABA by enzyme-linked immunosorbent assay (ELISA) with GA and ABA ELISA Kits, following the instructions provided by the manufacturer (Shanghai Youxuan Biological Technology Co., LTD). We measured absorbance at 450 nm using an ELISA Reader.

Seedling emergence experiment

To compare germination of *T. sebifera* seeds from the two ranges, we conducted an experiment in an unheated greenhouse with a glass paneled roof and mesh covered sides at Wuhan Botanical Garden, Chinese Academy of Sciences between April and May 2018. The greenhouse conditions were similar to the ambient environment at 40–70% relative humidity, 13/11 h light/dark cycle, and 26°C during the day and 16°C at night. We sowed 30 seeds from each of 24 populations collected in 2017 in ten 72-cell seedling trays (12 populations per range, Supplementary Table S2). The cell in each tray was 4 cm in deep, 16 cm² in area, and filled with peat soil (Huayu peat soil, Zhenjiang Peilei Organic Fertilizer Co., Ltd., China). In each tray, three seeds from each population were planted into three randomly selected cells (2 cm deep). During the experiment, we watered the seeds with tap water and checked seedling emergence every day. Furthermore, we randomly rearranged the position of trays every week to reduce the possible effects of environmental heterogeneity within the greenhouse. We ended the experiment when no additional seedlings emerged for seven consecutive days.

To examine the impact of study site on seed germination (i.e. to examine whether relative germination rates depended on our specific environmental conditions in China), we conducted an additional experiment from April to May 2018 in a greenhouse at Rice University (Houston, TX) using seeds collected in 2017 (12 populations per range, Supplementary Table S2). Greenhouse conditions were 50–80% relative humidity, 14/10 h light/dark cycle, and 28°C during the day and 16°C at night. Furthermore, to examine the impact of year in which seeds were collected on seed germination (i.e. to tease apart phenotypic vs. genetic variation among populations), we used seeds collected in both 2016 and 2017 (seven populations per range of each year, Supplementary Table S2). We used the same protocol to prepare and plant seeds as we did in the China greenhouse but used a different soil (organic potting soil, Espoma Company, Millville, NJ) and only recorded final germination.

Statistical analyses

To examine the differences in seed physical (volume, mass, coat hardness and coat thickness) and chemical properties (GA, ABA, GA:ABA, crude fat, soluble protein and sugar) between introduced and native population origins, we performed ANOVAs (proc mixed) with seed origin (introduced vs. native) as the fixed effect and seed populations nested within each origin as the random effect. Furthermore, to examine the differences in seed physical and chemical properties among populations within each range, we performed separate ANOVAs for seeds collected in China versus USA with population as a fixed effect.

To examine the differences in seed germination traits (day of first germination, days to 50% of total seed germination and germination rate) between introduced and native population origins, we performed ANOVAs (proc mixed) as described above. We also used a repeated measures ANOVA to test the odds of a seed germinating (ln [probability of a seed germinating / probability of a seed not germinating]) on seed origin, populations nested within origin, time and origin × time (proc glimmix, binomial distribution, logit link). We used an adjusted means partial difference test to determine whether germination odds differed for introduced versus native population origin seeds on different days. To examine the differences in seed germination rates and timing among populations within each range, we performed separate ANOVAs for seeds collected in China versus USA with population as a fixed effect.

To examine the relationships between seed physical (seed volume) and chemical (GA, GA:ABA and fat) properties with days to 50% of total seed germination, we performed Pearson correlations (proc corr). The selected seed parameters were significantly different between introduced and native origins. Native, introduced and all populations were analyzed separately.

To examine the effects of study sites on seed germination, we performed Pearson correlations for the percent germination of populations of 2017 collected seeds in the China versus US greenhouses. To examine the effects of year in which seeds were collected, we performed Pearson correlations of 2017 versus 2016 collected in the US greenhouse.

All data were analyzed using SAS 9.4 (SAS Institute, Inc., Cary, NC).

RESULTS

Seed physical properties

Seeds from the introduced range were larger than those from the native range ($F_{1,22} = 37.09$, $P < 0.001$, Fig. 1a), but they did not differ in mass ($F_{1,22} = 3.69$, $P = 0.068$, Fig. 1b), coat thickness ($F_{1,19} = 1.46$, $P = 0.242$, Fig. 1c) or hardness ($F_{1,22} = 1.46$, $P = 0.240$, Fig. 1d).

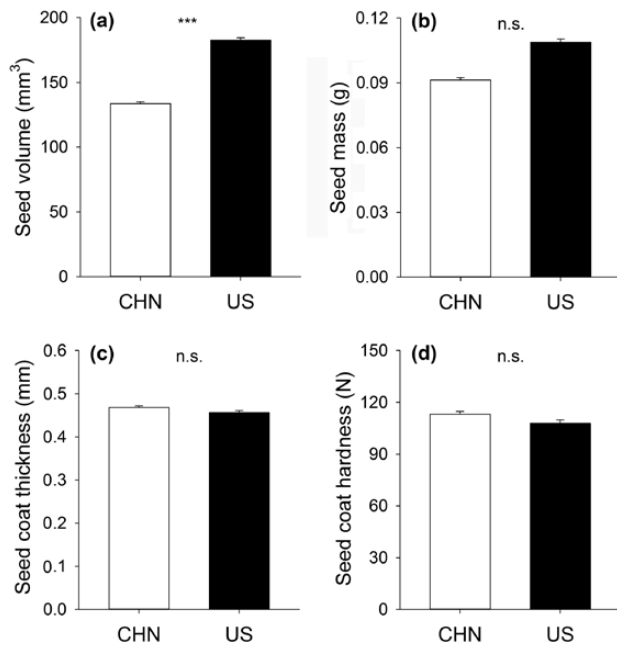


Figure 1: Physical properties of *Triadica sebifera* seeds collected from introduced (USA, black) and native (China, white) ranges. (a) Volume, (b) mass, (c) coat thickness, (d) coat hardness. Seed coat hardness was measured using seed break force. Seed sources, collection times and number of replicates are given in [Supplementary Tables S1](#) and [S2](#). Means + 1 SE are shown. Differences between origins were determined by nested ANOVAs with origin as fixed effect and populations as random effect nested within each origin.

Furthermore, all physical properties were significantly different among introduced (all $P < 0.001$) and native populations (all $P < 0.01$, [Supplementary Table S3](#)).

Seed chemical properties

The kernel GA content of seeds from the introduced range was significantly higher than that of seeds from the native range ($F_{1,22} = 7.76$, $P = 0.011$, [Fig. 2a](#)). Kernel ABA content did not vary between introduced and native ranges ($F_{1,22} = 0.04$, $P = 0.843$, [Fig. 2b](#)), so GA:ABA was greater for seeds from the introduced range ($F_{1,22} = 5.46$, $P = 0.029$, [Fig. 2c](#)). In contrast, the kernel crude fat content of seeds from the native range was significantly higher than that of seeds from the introduced range ($F_{1,22} = 9.46$, $P = 0.006$, [Fig. 2d](#)). However, there was no significant difference in soluble protein ($F_{1,22} = 1.00$, $P = 0.328$, [Fig. 2e](#)) or sugar ($F_{1,22} = 0.00$, $P = 0.997$, [Fig. 2f](#)) content in seed kernels between introduced and native ranges. Furthermore, all chemical properties were significantly different among introduced (all $P < 0.001$) and native populations (all $P < 0.001$, [Supplementary Table S3](#)).

Seedling emergence traits

In the China greenhouse, the day of first germination for seeds did not depend on seed origin ($F_{1,21} = 0.10$, $P = 0.761$, [Fig. 3a](#)), but it took ~3 fewer days for seeds from the introduced range to have 50% of their total seed germination compared to those from the native range ($F_{1,21} = 9.69$, $P = 0.005$, [Fig. 3b](#)). At the end of the experiment, germination rate for seeds from the introduced range was significantly lower than for those from the native range ($F_{1,22} = 15.04$, $P < 0.001$, [Fig. 3c](#)). The overall odds of a seed germinating did not vary with seed origin ($F_{1,22} = 2.20$, $P = 0.152$), but they did depend on population ($F_{20,594} = 6.5$, $P < 0.001$, [Fig. 4a](#)). Seed germination

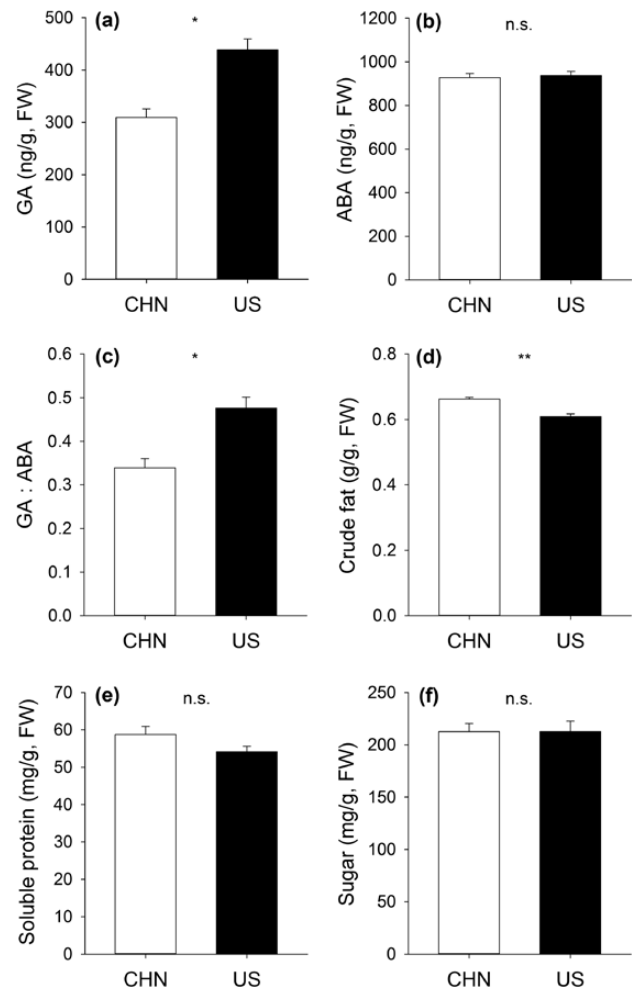


Figure 2: Chemical properties of seed kernels of *Triadica sebifera* collected from introduced (USA, black) and native (China, white) ranges. (a) Gibberellins (GA), (b) abscisic acid (ABA), (c) the ratio of GA to ABA, (d) crude fat, (e) soluble protein, (f) sugar. Seed sources, collection times and number of replicates are given in [Supplementary Tables S1](#) and [S2](#). Means + 1 SE are shown. Differences between origins were determined by nested ANOVAs with origin as fixed effect and populations as random effect nested within each origin.

odds varied with time ($F_{27,594} = 4.1$, $P < 0.001$) and the interaction of population and time ($F_{27,594} = 3.0$, $P < 0.0001$). Seeds from the introduced range had higher odds of germination earlier but lower odds of germinating later compared to the dynamics of those from the native range ([Fig. 4b](#)).

Furthermore, populations with larger seeds achieved 50% of total germination more quickly ($r = -0.447$, $P = 0.033$, [Fig. 5a](#)). Although not significant, seed GA ($r = -0.326$, $P = 0.120$) and GA:ABA ($r = -0.297$, $P = 0.169$) were negatively correlated to days to achieve 50% of total germination ([Fig. 5b](#) and [c](#)). The crude fat of seeds was not correlated with days to achieve 50% of total germination ($P = 0.535$, [Fig. 5d](#)). There was no correlation between seed properties and seed germination traits among introduced or native populations (all $P > 0.2$).

The germination rate in China versus US greenhouses for seeds collected in 2017 were strongly positively correlated ($r = 0.713$, $P < 0.0001$, [Supplementary Fig. S1a](#)). However, seed germination rates in the US greenhouse were not correlated for seeds collected in 2016 versus 2017 ($P = 0.987$, [Supplementary Fig. S1b](#)).

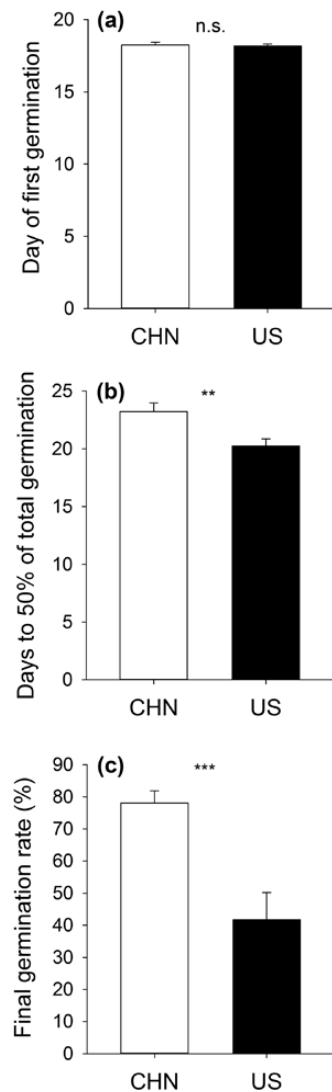


Figure 3: Seed germination traits of *Triadica sebifera* seeds collected from introduced (USA, black) and native (China, white) ranges. (a) Day of first germination, (b) days to 50% of total germination, (c) germination rate. Seed sources, collection times and number of replicates are given in Supplementary Tables S1 and S2. Means + 1 SE are shown. Differences between origins were determined by nested ANOVAs with origin as fixed effect and populations as random effect nested within each origin.

DISCUSSION

Differences in seed germination potentials between introduced and native populations of invasive plants have been demonstrated in some invasive plant species, including *U. pumila* (Hirsch *et al.* 2012) and *Ulex europaeus* (Udo *et al.* 2017). Our study provides clear evidence that introduced populations of *T. sebifera* had lower seed germination rates than native ones. This negative effect of lower germination rate may be attenuated because of greater seed productivity of introduced populations than native ones (Siemann and Rogers 2001). Furthermore, we found that *T. sebifera* introduced populations had comparable germination starting time, but germinated faster than native ones. For some invasive plants, quick germination is crucial (Gioria and Pyšek 2017), and a little variation at the beginning of a plant's growth may have large effects by the end because of two potential mechanisms. In the case of resource competition, early germinating of some invasive plants can exploit resources immediately and reduce the resources

available for surrounding native plants. Previous studies have found that *T. sebifera* has superior competitive ability for light and soil resources compared with native species (Rogers and Siemann 2002; Siemann and Rogers 2003). On the other hand, early germination of some invasive plants can release secondary metabolites and inhibit seed germination and seedling growth of surrounding native plants. However, experimental evidence indicates that this is not important for *T. sebifera*'s success as an invader (Paudel *et al.* 2014; Rua *et al.* 2008), so differences in germination timing are more likely to be important for introduced populations of *T. sebifera* in resource competition with native species.

Seed size (mass or volume) commonly varies between and within species, even within a single plant, can be influenced by genetics and reflects maternal investment and environmental conditions (Harper *et al.* 1970; Nonogaki 2006). There are numerous examples where invasive plant seed size increases from native to introduced ranges or where seeds of invasive species are larger and heavier than that of non-invasive congeners (Buckley *et al.* 2003; Daws *et al.* 2007; Graebner *et al.* 2012). For example, the seeds of the invasive plant *Centaurea solstitialis* from the invaded range in California were larger than those from the native range in Spain, while the non-invasive species of *Centaurea calcitrapa* and *Centaurea sulphurea* did not show a trend of increasing size in non-native ranges (Graebner *et al.* 2012). Our results were also consistent with this trend of increasing seed volume in the introduced range, though this may reflect genetics and/or environmental factors. Although there are counter examples (Souza and Fagundes 2014), for many species larger seeds germinate faster than smaller ones (Dunlap and Barnett 1983; Leverett and Jolls 2014; Suárez-Vidal *et al.* 2017) which is consistent with our results. The seed coat is a primary defense against adverse environmental conditions but also a barrier impeding radicle emergence even under suitable germination conditions. We found no differences in *T. sebifera* seed coat thickness or hardness between introduced and native populations, so those physical traits likely do not explain differences in *T. sebifera* seedling emergence.

Inside viable seeds, seed reserves, such as proteins, lipids and carbohydrates need to be mobilized prior to use. These materials are usually enough for germination development, and their levels are affected by the process of germination (Elamrani *et al.* 1992; El-Adawy *et al.* 2003; Suda and Giorgini 2000). However, the material and energy resources in seeds are linked to early growth and also have significant effects on seedling vigor and survival (Elamrani *et al.* 1992; Mtambalika *et al.* 2014). For example, seeds of *C. solstitialis* from their introduced US range were found to have a higher starch content than those from the native range in Eurasia, resulting in higher biomass of introduced populations after 2 weeks of growth (Widmer *et al.* 2007). In this study, however, there were no significant differences in soluble proteins or sugars in the seed kernels at the beginning of germination between introduced and native *T. sebifera* populations, which implies the availabilities of those two reserves were not different. On the other hand, the crude fat content of seed kernels from the native populations was higher than that of those from introduced populations, although this higher value of crude fat in seeds from native populations may be offset by its lower volume seeds. In water-soaked seeds during germination, many kinds of enzymes are essential for mobilizing and using the seed reserves and the activities of those enzymes are related to germination time (Betty and Finch-Savage 1996). However, we did not measure these activities of the mobilizing enzymes in imbibed seeds of *T. sebifera*, something that should be determined in a further germination study.

Physiological state, controlled by plant hormones, mainly determines whether or not seeds germinate. Seed dormancy and germination are influenced by nearly all plant hormones and their interactions, but it is now generally accepted that ABA and GAs are the leading regulators (Liu *et al.* 2011; Shu *et al.* 2016; Yang *et al.* 2015). The balance of GA

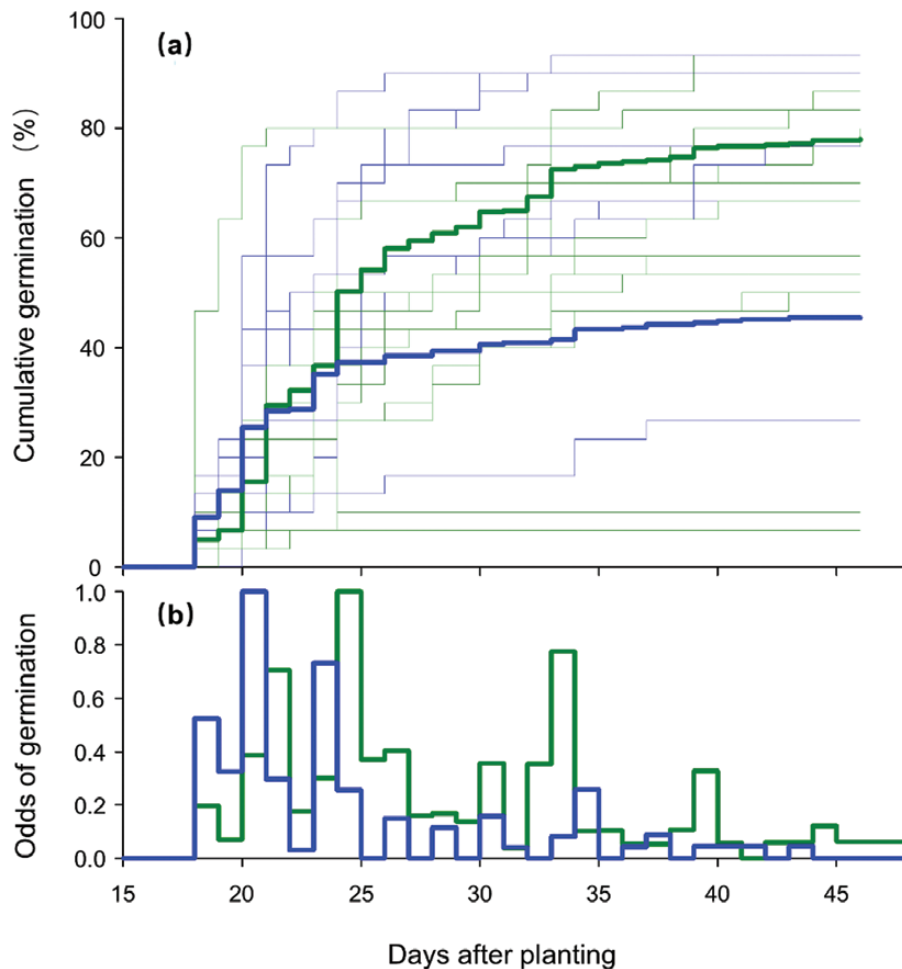


Figure 4: Germination of *Triadica sebifera* seeds collected from introduced (USA, blue) and native (China, green) ranges. **(a)** The cumulative number of seeds germinating for introduced and native populations over the experiment. Thin lines indicate individual populations and the thick lines indicate the average values for introduced versus native populations. **(b)** The odds (\ln [probability of a seed germinating / probability of a seed not germinating]) of a seed germinating over the experiment for introduced versus native populations (back-transformed from log odds ratios). The day with the maximum germination odds for introduced or native populations is set to one. Seed sources, collection times and number of replicates are given in [Supplementary Tables S1 and S2](#).

and ABA determines the fate of a seed: low GA and high endogenous ABA levels result in deep seed dormancy and low emergence, while high GA and low ABA levels promote seed germination (Liu *et al.* 2011; Shu *et al.* 2016). A higher GA/ABA ratio in the embryo can stimulate seeds to germinate faster (Yang *et al.* 2015). Shah *et al.* (2018) found that proanthocyanidins (PAs) in *T. sebifera* seed coat and endospermic cap could inhibit seed germination while seed coat extract could upregulate the transcription level of dormancy-related genes, GA suppressing genes, plus ABA biosynthesis and signaling genes. These findings suggest that proanthocyanidins (PAs) are involved in the expression of hormone GA and ABA regulators. While one of our important results is that the GA content and the GA/ABA ratio of *T. sebifera* seeds from introduced populations were significantly higher than those of seeds from native populations, no significant difference in ABA content was found between the two origins. The higher GA content and the higher GA/ABA ratio in seeds from introduced populations of *T. sebifera* may help to explain the difference in timing of seedling emergence between introduced and native populations.

We do not have data on the germination timing or properties of seeds produced by trees in 2016, but the germination rate data suggest an important role for phenotypic plasticity in seed germination. Specifically, the lack of a correlation between the germination of a

population's 2016 seed crop and its 2017 seed crop indicate that the quality of seeds produced by a population is inherently variable or that differences in environmental conditions (such as weather or natural enemies) among years have an important effect on seed quality via a plastic, phenotypic response. However, the strong, positive correlation between germination rates of 2017 seeds in the China and US greenhouse indicate that a set of seeds has a germination rate that is expressed in similar ways in two somewhat different sets of conditions. It is important to note that our study simulated the most common mode (passage through a bird) and timing (winter) of dispersal and so additional studies with other scenarios would be helpful because each can impact tallow tree seed germination (Park *et al.* 2012; Renne *et al.* 2001). Disentangling the roles of plasticity and genetic variation in the characteristics and germination of seeds of introduced and native plants is also an important remaining research question.

In summary, we show that seeds of introduced populations of *T. sebifera* germinated faster than those from native ones and this difference may reflect their larger volume and/or higher GA content in the kernel. The faster germination may play a role in its invasion by reducing resource availability to surrounding native plants. Thus, linking seed germination traits to invasion success is an important future step to better understand invasion mechanisms and inform

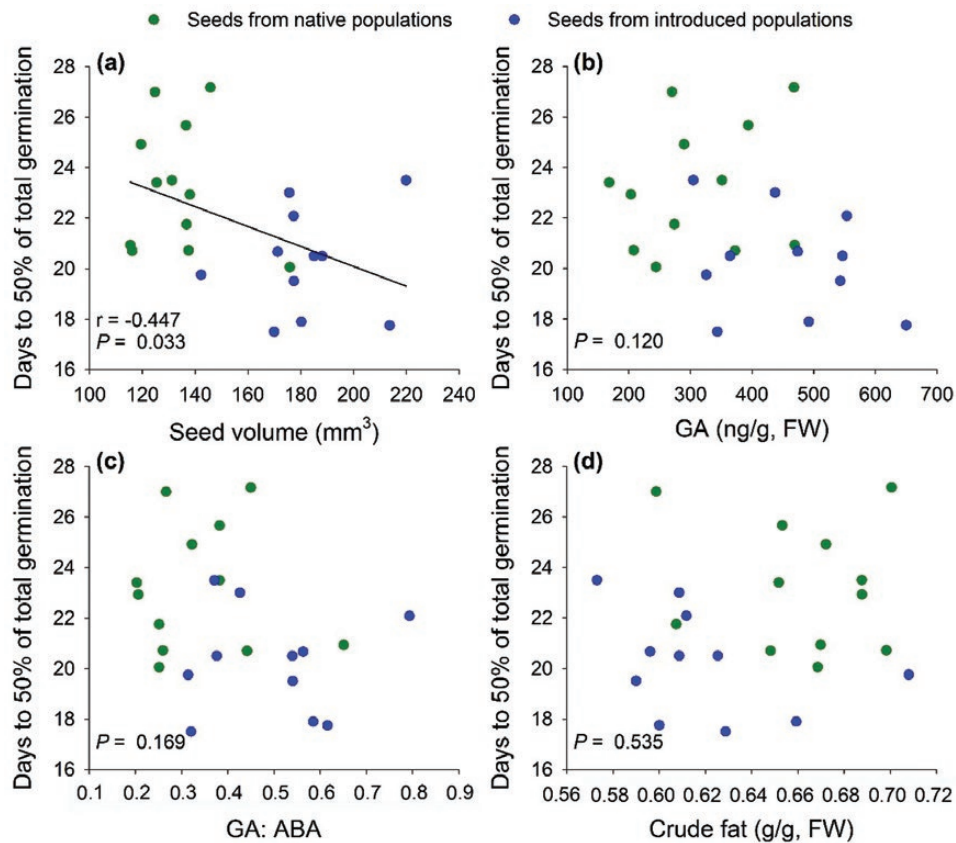


Figure 5: The relationship between days to 50% of total germination and seed properties. (a) Seed volume, (b) GA concentration, (c) GA:ABA ratio, and (d) crude fat in the seed kernel. Each point indicates an introduced (blue) or native population (green). Seed sources, collection times and number of replicates were given in Supplementary Tables S1 and S2. The *r* and *P* values are from Pearson correlations. The solid line represents a significant linear correlation.

invasive plant management at the establishment stage for *T. sebifera* and other invasive plants that reproduce by seeds.

Supplementary Material

Supplementary material is available at *Journal of Plant Ecology* online. Table S1: Locations and characteristics of seed collection sites and times for 24 populations of *Triadica sebifera* used in this study. Table S2: Seed sources, collection times and number of replicates for measurements of physical and chemical properties of *Triadica sebifera* as well as in germination experiments. Table S3: One-way analyses of variance (ANOVAs) for physical and chemical properties of *Triadica sebifera* seeds among populations within native and introduced ranges. Figure S1: The impacts of study site and seed collection time on seed germination.

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