Chemical responses of an invasive plant to herbivory and abiotic environments reveal a novel invasion mechanism

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HIGHLIGHTS
• Invasive tallow trees had higher foliar tannins at latitudes with lower herbivory.
• Tallow trees had higher foliar flavonoids in harsher abiotic environments.
• They may trade off chemical responses to herbivory and abiotic environments.
• Their chemical responses to changing environments may facilitate their invasion.

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ABSTRACT
Invasive plant environments differ along latitudes and between native and introduced ranges. In response to herbivory and abiotic stresses that vary with latitudes and between ranges, invasive plants may shift their secondary chemicals to facilitate invasion success. However, it remains unclear whether and how invasive plant chemical responses to herbivory and chemical responses to abiotic environments are associated. We conducted large scale field surveys of herbivory on the invasive tallow tree (Triadica sebifera) along latitudes in both its native (China) and introduced ranges (United States) and collected leaf samples for analyses of tannins and flavonoids. We used data on climate and solar radiation to examine these chemical responses to abiotic environments and their variations along these latitudes and between ranges. We also re-analyzed previously published data from multiple common garden experiments on tallow tree to investigate genetic divergence of secondary chemical concentrations between introduced and native populations. We found foliar tannins and herbivory (chewing, sucking) were higher in the native range compared to the invasive range. Allocation to tannins versus flavonoids decreased with latitude in the native range but did not vary in the invasive range. Analyses of previously published common garden experimental data indicated genetic divergence contributes to chemical concentration differences between ranges. Our field data further indicated that the latitudinal patterns were primarily phenotypic responses to herbivory in China while in US they were primarily phenotypic responses to abiotic environments. The variation of tannins may be linked to flavonoids, given tannins and flavonoids share a biosynthesis pathway. Together, our results suggest that invasive plants adjust their secondary metabolism to decrease
1. Introduction

In response to shifting environments, plants may change their traits through evolutionary (genotypic) (Agrawal et al., 2015; Colautti and Barrett, 2013; Züst et al., 2012) or ecological strategies (such as phenotypic plasticity) (Castillo et al., 2014; Liu et al., 2016; Riis et al., 2010). These strategies enable plants to adapt to varying abiotic and biotic environments at a biogeographic scale such as introduced and native ranges (Sakata et al., 2017; Siemann et al., 2017; van Boheemen et al., 2019) and a range of latitudes (Bhattarai et al., 2017; Castillo et al., 2014; Pratt et al., 2014). When adapting to varying biotic environments (such as herbivory regimes) or abiotic environments, plants often produce distinct types and amounts of secondary chemicals (Abdala-Roberts et al., 2016; Moreira et al., 2014). To date, however, it remains unclear whether and how invasive plant chemical responses to herbivory are associated with their chemical responses to abiotic environments.

Because plant resources are limited and physiological processes may be closely associated (Coley et al., 1985; Herms and Mattson, 1992), chemical responses to abiotic and biotic environments may not be independent (Dofosz et al., 2018). This in turn may determine the identities and amounts of secondary chemicals. Therefore, simultaneous consideration of invasive plant chemical responses to abiotic and biotic environments could help to fully understand invader adaptive and ecological strategies. This is particularly important for secondary chemicals that have multiple functions. For example, tannins can help plants defend against herbivores (Berenbaum, 1983; Xiao et al., 2019a) and could also help plants tolerate many abiotic factors, such as temperature (Graglia et al., 2001) and precipitation (Abdala-Roberts et al., 2018; Moreira et al., 2017). Similarly, flavonoids could enhance plant resistance against herbivores and microbial pathogens (Mathesius, 2018), and play a role in plant tolerance to UV, drought and salinity (Mathesius, 2018; Tattini et al., 2004) as well. Therefore, testing simultaneous responses of secondary chemicals to multiple factors is critical for understanding the secondary chemical strategies of plants. Specifically, a biogeographic approach that includes broad latitudinal gradients in multiple continental ranges may help to reveal how invasive plants use different chemical allocation patterns in varying environments.

Many invasive plants escape co-evolved natural enemies and experience lower herbivory in their introduced ranges, leading to vigorous growth (Enemy Release Hypothesis) (Elton, 1958). As a result, invasive plants may evolve higher competitive ability by allocating more resources to growth/reproduction and less resources to herbivore defense (Evolution of Increased Competitive Ability Hypothesis) (Blossey and Nötzold, 1995; Joshi and Vrieling, 2005). Moreover, because herbivore abundance, community composition, and damage can differ along latitudes (Camacho and Kotanen, 2014; Moles et al., 2011; Moreira et al., 2015), plants of both introduced and native populations of an invasive species are expected to show latitudinal variation in chemical responses to herbivory. In addition, abiotic environmental factors, such as climate and solar radiation (including UV) often vary along latitudes (Caldwell et al., 1989; Garibaldi et al., 2011), potentially affecting plant traits in both native and introduced ranges (Colautti et al., 2017; Xiao et al., 2019b). Thus, we predict that, along latitudinal gradients, plants of native and introduced populations may differ in both adaptation to abiotic environments and herbivory, likely resulting in different responses of secondary chemicals to these factors. For example, in native ranges, plants may show a latitudinalcline of defense to herbivory; however, as a result of lack of specialist herbivores in introduced range, invasive populations may show little variation in defense levels along latitudes as variation in herbivory is smaller. Moreover, as chemical responses to abiotic and biotic environments may not be independent, invasive plant chemical responses to herbivory and chemical responses to abiotic factors may be closely associated, in particular for chemicals that share a biochemical synthesis pathway. For example, hydrolysable and condensed tannins are synthesized upstream or downstream of the flavonoid biosynthetic pathway (Chen et al., 2009; Hichri et al., 2011; Vogt, 2010). Thus, the changes of tannins may be associated with changes in flavonoids (Huang et al., 2013), for example, tannins in Betula nana increased with increasing temperature and herbivory, while flavonoids decreased (Graglia et al., 2001), and Betula pubescens had high flavonoids but low tannins when exposed to high light and low herbivory (Wam et al., 2017). Though previous studies have reported the chemical responses of invasive plants to changes in herbivores (e.g., specialists vs. generalists) (Huang et al., 2010; Wang et al., 2012) or climate (Sampaio et al., 2016; Xiao et al., 2019b), to date, however, no study has examined the simultaneous response to these factors in a single study. Such knowledge is critical for a full understanding of the mechanisms of plant invasion success.

In this study, we examined foliar secondary chemicals (tannins and flavonoids) of tallow tree (Triadica sebifera (L.) Small, Euphorbiaceae) (hereafter ‘Triadica’) along latitudes in both its native (China) and introduced (US) ranges. We investigated the effects of biotic and abiotic factors associated with latitude on production of these chemicals. Previous studies have shown that Triadica plants from native populations have higher resistance to specialist herbivores than those from introduced populations, apparently due to higher content of tannins (Huang et al., 2010; Siemann and Rogers, 2001; Wang et al., 2012). Furthermore, Triadica plants from introduced populations have high performance in a range of abiotic stresses (Yang et al., 2015), perhaps as a result of their higher content of flavonoids (Wang et al., 2012). In this study we did field surveys of herbivory and used field-collected leaves for these chemical analyses. We re-analyzed previously published common garden data of US and China populations to investigate contributions of genetic differences to chemical concentration variation. We also investigated the relationships of temperature, precipitation, and solar radiation with these chemicals. Specifically, we asked: (1) What are the latitudinal patterns of herbivory and secondary chemicals in the introduced vs. native ranges? (2) Are these patterns caused by genetic and/or phenotypic mechanisms? (3) What biotic or abiotic factors contribute to latitudinal patterns of chemicals in the introduced and native ranges and (4) How are secondary chemical responses to herbivory and secondary chemical responses to abiotic environments in both ranges related? Results of this study will advance our knowledge of how invasive plants respond to biotic and abiotic factors independently and jointly in their native and introduced ranges.

2. Material and methods

2.1. Study system

Triadica is a deciduous tree that can reach up to 15 m in height. It is native to Asia and widely distributed in sparse forests, edges of crop fields and near bodies of water of southern China (Zhang and Lin, 1994; Zheng et al., 2005). Now it has become one of the most pervasive invasive tree species aggressively displacing native plant species, invading coastal prairies, floodplains, riparian areas, wetlands and the lake margins of forest lands, and agricultural areas in the southeastern United States (Bruce et al., 1997; Gan et al., 2009; Pile et al., 2017).
Previous studies have shown that, relative to native populations, plants of the introduced populations have faster growth (Siemann and Rogers, 2001; Zhang et al., 2019), lower defense to specialists and generalist herbivores (Huang et al., 2010; Wang et al., 2012), higher herbivore tolerance (Carrillo and Siemann, 2016; Wang et al., 2016b; Zou et al., 2008) and higher plasticity in the response to abiotic stressors (Carrillo and Siemann, 2016). *Triadica* plants from introduced populations in US outperform *Triadica* plants from native populations in China along gradients of various abiotic stresses, such as low light (Wang et al., 2011), drought (Butterfield et al., 2004), flooding (Butterfield et al., 2004), salinity (Chen et al., 2013), and UV (Wang et al., 2016a).

2.2. Field survey and sampling

In 2015, we conducted a geographical survey in the native range at 22 locations spanning 14 degrees of latitude from 19.38° to 34.05° N in central and southern China (Table S1, Fig. 1). Based on herbivore phenomenology, we started field surveys in the southernmost location (Hainan Province) in early July (2015.07.04) and ended in the northernmost location (Henan Province) in late July (2015.07.30). In total, we sampled 209 plants with sample sizes ranging from 3 to 15 individuals per location (Table S1). In 2016, we conducted a survey in the introduced range at 18 locations spanning 6 degrees of latitude from 28.23° to 34.25° N in the southeastern United States (Table S2, Fig. 1). We started field surveys in late July (2016.07.21), completing all sampling within one week. In total, we sampled 171 plants, with sample sizes ranging from 6 to 12 individuals per location (Table S2). Habitat types included undisturbed areas, abandoned fields, roadsides, forests and grassland edges (Table S1 and S2). Our samples spanned the entire latitudinal distribution in both China and the US.

We observed and sampled *Triadica* individuals with an average height of 2 m (1.5 to 2.5 m), and within each location, the distance between each sampled tree was more than 10 m. For each plant, we randomly selected 6 branches and recorded the numbers of young leaves undamaged and damaged by insects. Then, we collected and scanned all the damaged leaves and put the undamaged leaves into a ziploc bag filled with silica gel. We dried all the undamaged leaf samples at room temperature. We brought these samples from the USA to China, and ground the samples (one per plant) using a ball mill and stored them in sealed tubes at −20 °C until chemical analysis.

Secondary chemicals in undamaged leaves may reflect plant constitutive ability to resist abiotic or biotic stresses. Thus, sampling and analyzing young and undamaged leaves could help reduce variation in defenses caused by site-specific induction of plant defenses (Abdala-Roberts et al., 2016), though damage on neighboring leaves may trigger systemic defense induction that affects secondary chemicals of undamaged leaves.

2.3. Herbivory measurements

We quantified herbivory on each plant in three ways: (1) leaf area damaged by chewing, (2) leaf rolling frequency and (3) abundance of sucking herbivores (none present in the USA; Hartley et al., 2010). For leaf area damaged, we first redrew the leaf margin if it was damaged. Then we used the image analysis software ImageJ (National Institutes of Health, Bethesda, MD, USA) to estimate the damaged area and total area of each leaf. We calculated chewing damage area (%) of each plant as: (removed leaf area of damaged leaves) / (total leaf area of damaged leaves) * 100 * (number of damaged leaves) / (total number of leaves). For leaf rolling frequency, we counted the number of leaves with leaf rolling damage and divided it by the total number of leaves on the branches. For sucking herbivores, we counted the number of sucking herbivores.

2.4. Chemical analyses

We analyzed total tannins and five individual flavonoids (quercetin, isoquercetin, quercitrin, kaempferitrin and kaempferol) content for each plant. We extracted a 100 mg sample with 5 ml of methanol-0.4% phosphoric acid in water solution (48:52, v: v) and removed insoluble material by filtering the solutions through a 0.22-μm membrane. We used the modified radial diffusion assay (Hagerman, 1987) to estimate the total tannin content. For the five flavonoids, we used high-performance liquid chromatography (HPLC) according to the methods in previous studies (Wang et al., 2012). We calculated the total flavonoid concentration of each plant as the sum of these five flavonoids.

2.5. Environmental variables

To account for the importance of abiotic factors to secondary chemicals of *Triadica* across latitudes in both China and the US, we downloaded free climate data for 1970–2000 from WorldClim - Global Climate Data (http://worldclim.org/version2) (Fick and Hijmans, 2017). We selected variables potentially important for plant secondary chemicals including annual mean temperature (avgT), maximum temperature of warmest month (MaxT), total annual precipitation (precip), and solar radiation (Srad). Other temperature variables (such as minimum temperature and growing degree days) were strongly correlated with average temperature (r > 0.9) and so were not included.

2.6. Statistical analyses

2.6.1. Effects of population origin and latitude on herbivory, secondary chemicals and climate variables

We used mixed model regressions to examine the effects of latitude on foliar tannin and flavonoid concentrations and their ratio (T:F; the relative change in tannins and flavonoid) for China and US sites while controlling for the non-independence of trees from the same site by including a random effect for population. Because some data appeared to have non-linear relationships with latitude, we fit both linear models and 2nd order polynomial models for each continent. We selected the best fitting model based on Akaike’s Information Criterion (AIC) values. We performed a similar set of analyses to examine the dependence of leaf area consumed, percent of leaves with rolling, and the abundance of sucking herbivores (all square-root transformed to meet the assumption of normality). To examine the possibility that significance of patterns were sensitive to multiple variables having been measured on each tree, we performed an additional multivariate mixed model regression with response variables tannins, flavonoids, leaf area consumed, leaves rolled and sucking herbivore abundance and the fixed predictors variable identity, variable identity×latitude and variable identity×latitude2 and the random effect of population. We used linear regressions to examine the effects of latitude on average temperature, maximum temperature, precipitation and solar radiation in China and US sites.

2.6.2. Environmental effects on chemicals

We conducted an additional set of regressions to simultaneously examine the effects of latitude (including a quadratic term), environmental factors (avgT, MaxT, precip, Srad), and herbivores (chewing, rolling, sucking) on flavonoids, tannins and their ratio in China and the US.

2.6.3. Genetic divergence and secondary chemicals

We re-analyzed our previously published data to investigate: 1) whether *Triadica* populations from China and the US differed in their foliar flavonoid and tannin concentrations when grown in common gardens, and 2) whether the foliar flavonoid and tannin concentrations depended on the latitude of the population where the seeds were collected in China or the US. These data were obtained from five studies that reported concentrations of foliar tannins and/or...
flavonoids measured for *Triadica sebifera* plants of control group (without any herbivory treatment) for multiple populations from China and USA that were grown in a common garden (under identical environmental conditions) (both flavonoids and tannins: Li et al., 2016; Wang et al., 2012; Wang et al., 2016b; tannins only: Huang et al., 2014; Wang et al., 2018; Fig. 1, Table S3). At least one of us was an author on each
of the 5 studies so we had access to the original data. We calculated Hedges g values for the effect of plant origin (China vs. US populations) and constructed fixed effect 95% confidence intervals (Borenstein et al., 2009). We calculated Fisher’s z values using r values, constructed fixed effect 95% confidence intervals, and back-converted them to r values for figures (Borenstein et al., 2009). We considered an effect to be significant when the 95% CI did not overlap zero.

As the ranges of Triadica in field survey and common gardens differed, we conducted an additional set of analyses of latitude effects on secondary chemicals and overall differences in tannins and flavonoids between the native and invasive ranges for field survey data using the same latitude spans as in the common garden datasets (China:24–34.5°, US:29–32.5°). All analyses were conducted using SAS version 9.4.

3. Results

3.1. Secondary chemicals, herbivory and climate along latitude in US and China

Foliar flavonoid concentrations did not differ significantly between the introduced and native ranges (Fig 2A). But they showed different types of latitudinal patterns between ranges as they decreased with increasing latitude for US sites and were highest at intermediate latitudes for China sites (Table 1, Fig 2B). Foliar tannin concentrations were higher on average in China than in US (Fig 2C). Similar to flavonoids, tannins decreased with latitude for US sites, but were highest at intermediate latitudes for China sites (Table 1, Fig 2D). The ratio of tannins to flavonoids were also higher on average in China than in US (Table 1, Fig 2E), and it decreased at higher latitudes in China sites but did not vary for US sites (Fig 2F).

Chewing herbivore damage was higher on average in China and at every latitude than in the US (Fig 3A). It was highest at intermediate latitudes for China sites and it did not vary with latitude for US sites (Table 1, Fig 3B). Rolling herbivore damage did not differ significantly on average between US and China sites (Fig 3C) and it did not vary with latitude in the US or China (Table 1, Fig 3D). Sucking herbivores were more abundant in China (none in the US, Fig 3E) and they did not vary with latitude in China (Table 1, Fig 3F).

In the multivariate model, variables differed on average (F5,669 = 2.65, P = 0.0219), and depended on variable identity×latitude (F5,669 = 2.44, P = 0.0333), variable identity×latitude (F5,669 = 2.38, P = 0.0375), and population (z = 2.36, P = 0.0092).

Average temperature, maximum temperature, and solar radiation each decreased with increasing latitude in both ranges but precipitation only decreased in China sites (Fig 5, Table S4).

In the regressions with latitude, environmental factors and herbivores, tannins depended positively on chewing damage in China sites but did not significantly depend on any variables in US sites (Table 2). Flavonoids did not vary with any of the predictors in the US or China. However, the ratio of tannins to flavonoids was significantly positively related to chewing damage and significantly negatively related to rolling damage in China but negatively related to solar radiation in the US (Table 2).

3.2. Genetic divergence of secondary chemicals between ranges

The meta-analysis of secondary chemicals results showed that plants from native China populations had significantly lower foliar concentrations of flavonoids and significantly higher foliar concentrations of tannins compared to plants from introduced US populations when they were grown in common gardens (Fig 4). For US populations, there was a significant but weak negative effect of latitude of seed collection on the concentrations of tannins (Fig 4). However, for China populations, there were no significant effects of latitude of seed collection on tannins (Fig 4). There was no significant effect of latitude of seed collection for US or China populations for flavonoids (Fig 4).

When we repeated the analyses for secondary chemicals of the field data using the narrower latitude range that corresponded to the latitude span in the common garden data, flavonoids no longer varied with latitude (China P = 0.73; US P = 0.38) but tannins (P < 0.0001) and T:F (P < 0.0001) decreased with latitude in China. Chewing damage no longer varied with latitude in China (P = 0.39).

4. Discussion

This study examined relationships among latitude, herbivory, climate and secondary chemical defense in Triadica across large latitudinal gradients in both its native and introduced ranges. We found higher herbivory and higher tannins for Triadica plants in the native range (China) than the introduced range (US), and that the two classes of chemicals (tannins and flavonoids) differed along latitudinal gradients in both US and China. Our meta-analysis of common garden experiments indicated strong genetic contributions to differences in these chemicals between ranges but not to variation with latitude. Rather, latitudinal patterns were more likely related phenotypic responses to varying herbivory and abiotic environments. Overall, these results suggest that the invasive Triadica adjusts its secondary metabolism to decrease chemicals that primarily defend against herbivory and increase those that help them to respond to their abiotic environment. Previous studies have separately reported how invasive plants adapt to biotic or abiotic environments by producing secondary chemicals (Bhattarai et al., 2017; Cronin et al., 2015; Joshi and Vrieling, 2005; Sampaio et al., 2016), but here, we show that, during the invasion process, the relative amount of the two chemical classes were closely related to herbivory (native range) or abiotic factors (introduced range) and these associations may play key roles in plant invasion success.

The “Latitudinal Biotic Interaction Hypothesis” posits that species diverseness is higher and biotic interactions are more intense at low than high latitudes (Schemske et al., 2009, Moles and Ollerton, 2016). However, in our study, the herbivory patterns along latitudes in both ranges did not support the Latitudinal Biotic Interaction Hypothesis (for similar inconsistent results see review by Moles et al., 2011; Kozlov et al., 2015; Anstett et al., 2016). Our analyses showed that tannins and the relative allocation to tannins vs. flavonoids were sensitive to herbivory in China. However, the relative allocation to tannins vs. flavonoids was sensitive to the abiotic factor solar radiation in the US but not to herbivory. This may be because invasive plants escaped natural enemies, leading to lack of selection for the Latitudinal Herbivory-Defense Hypothesis (which predicts that increasing herbivore pressure at lower latitudes should select for greater investment in plant defenses, LHDH, Coley and Aide, 1991).

Many phenolics are known to facilitate plants responding to abiotic environmental stresses such as climate and solar radiation (dos Santos Nascimento et al., 2015; Karpinnen et al., 2016). In this study, we found that higher solar radiation was associated with a greater relative allocation to flavonoids in the introduced range but, in contrast, there were no detectable abiotic effects on the variations of tannins, flavonoids or their ratio in China. These findings are consistent with a scenario in which biotic factors (herbivory) are relatively more important for the secondary chemical production of plants in their native ranges where herbivory is more intense but tolerance of abiotic stress is relatively more important in the introduced range where herbivory is less intense.

When comparing the differences between continents, we found solar radiation was the most important predictor for the ratio of tannins to flavonoids in US sites, with the relative amount of flavonoids increasing as latitudes and solar radiation decreased. Notably solar radiation levels are higher in US than China sites, suggesting US plants may invest relatively more in flavonoids to increase adaptation to higher solar radiation in the invasive range, as flavonoids could increase solar radiation tolerance (Jaakola et al., 2004; Tattini et al., 2005). However, in China sites, herbivory (chewing and leaf rolling) was the most important
predictor for the variations of both tannins and the ratio of tannins to flavonoids, suggesting the key role of herbivory in regulating chemical defense in the native range. In addition, the lower leaf chewing damage and lack of sucking herbivores in US sites supported the Enemy Release Hypothesis (Elton, 1958), which may thus contribute to the chemical differences between native and invasive ranges, i.e., tannins and the

**Fig. 2.** (A, B) Foliar flavonoids, (C, D) foliar tannins and (E, F) ratio of tannins to flavonoids of Triadica sebifera trees growing in the native (China, light bars) and introduced range (US, dark bars). **P < 0.01. Bars indicate means ± 1 standard error in A, C and E. Fitted curves and lines indicate the best model for that variable and continent in B, D and F (see Table 1 for details).**
The decreasing ratio of tannins to flavonoids were lower in US than in China. This result indicated that changes in herbivory level could drive changes in secondary chemicals during the invasion process. Moreover, the reductions in tannins and the ratio of tannins to flavonoids in the introduced range may reflect tradeoffs in the production of these classes of chemicals. Thus, to respond to varying environments plants may shift chemical metabolites in response to lower herbivory and higher solar radiation, which might enhance Triadica performance in the US.

Many invasive plants have genetic differences in chemical defenses between their native and introduced populations that increase their success with the novel herbivore communities they encounter in their new ranges (Caño et al., 2009; Joshi and Vrieling, 2005). This has been reported in previous studies on Triadica that show plants from US populations have lower tannins but higher flavonoids than those from China populations (Li et al., 2016; Wang et al., 2012; Wang et al., 2016b). Consistent with this, our meta-analysis results suggest that genetic differences contribute to the differences in secondary chemicals between ranges. However, our meta-analysis found only a weak negative effect of latitude of seed collection for tannins for US populations when plants from different latitudes grew under identical conditions. It should be noted that the latitudinal range for these populations was not as broad as the field surveys but other studies have found genetic divergence in similar latitudinal ranges (Pratt et al., 2014). The patterns of latitude of seed collection for tannins of China populations, flavonoids of US populations, and flavonoids of China populations grown under identical conditions were not significant though the median effect was always negative. This indicates that latitudinal gradients of secondary chemicals within a range only depend weakly or not at all on genetic differences. It is important to note, however, that the greater latitudinal range for the field sites vs. population origins in published common garden studies may have limited our ability to detect genetic vs. phenotypic contributions to latitudinal clines in secondary chemicals, especially with the reduced significance of latitudinal patterns in the field survey when only a subset of sites were analyzed. The results of the field study do suggest that factors that vary with latitude, such as herbivory and climate, may play a role in determining plant secondary chemical patterns within the introduced and native ranges via plastic phenotypic responses.

Many secondary chemicals within same groups share the same biosynthesis pathway (Salminen and Karonen, 2011), thus changes in one class of chemicals may lead to changes in another class of chemicals, causing interactive plant responses to multiple factors. In this study the decreasing ratio of tannins to flavonoids with increasing latitudes in China may suggest associations between the two chemicals affected by latitudes. Such tradeoffs between tannins and flavonoids were also reported among populations of dwarf birch, Betula nana and between trees of differing ages of downy birch, Betula pubescens (Graglia et al., 2001; Wam et al., 2017). Thus, even though tannins and flavonoids may both help plants respond to abiotic environments, plants may optimize their amounts and types of tannins and flavonoids to adapt to secondary chemicals in invasive plants have important implications in invasion biology. In the last decades, many studies on invasive plants have focused on how changes in herbivory during the invasion process contributed to the evolution of plant growth and defense (Allen et al., 2017; Bhattachari et al., 2017; Gruntman et al., 2017), or how invasive plants show plastic responses to abiotic environments to facilitate their invasion success (Riis et al., 2010; Xiao et al., 2019b). However, these studies are often independent, i.e., few studies simultaneously consider both plant defense to herbivory and plant tolerance to abiotic environments during the invasion process. In this study, we show Triadica plants of introduced and native populations differ in their secondary metabolisms: US populations invest relatively more in flavonoids at low latitudes, potentially increasing their tolerance to solar radiation and then enhancing their growth (Cao et al., 2011; Chebrolu et al., 2016; Deng et al., 2017), while China populations invest more in tannins which increase their defense to herbivory (Wang et al., 2012). Previous studies (Deng et al., 2017; Huang et al., 2010; Siemann et al., 2017; Wang et al., 2012; Yang et al., 2015) show that US populations have higher herbivore tolerance and faster growth than the China populations. Our results, together with these previous findings, indicate that invasive plants, such as Triadica, could trade off abiotic tolerance, growth, and chemical defense to herbivory. These novel findings may also apply to other invasive plants, highlighting the importance of physiological changes in mediating simultaneous plant responses to abiotic and biotic environments across the invasion process. More generally, a decrease in plant chemical defenses in response to enemy release, may lead to an increase in other chemicals. This allows them to be more adaptive in response to abiotic stresses, facilitating invasion success across heterogeneous environments.

We note that, in addition to herbivory, climate and solar radiation examined in this study, plant secondary chemicals can also be influenced by other abiotic and biotic factors such as soil nutrients and soil biota. In this regard, future field studies and common garden experiments controlling herbivores, pathogens and other biotic factors, as well as abiotic factors are needed. Such factor-controlled studies along latitudes in both native and introduced ranges of invasive plants could explicitly reveal the contributions of each factor to changing secondary chemicals, and to further disentangle genetic and environmental effects. Furthermore, molecular tools may be used to identify genetic divergence between invasive and native populations, for example, identifying loci that may undergo epigenetic changes regulating tannins and flavonoids.

### Table 1

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<th>Variable</th>
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<td>flav = 387.5 + 0.61 × lat</td>
<td>564.7</td>
<td>0.0001</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>Suck</td>
<td>China</td>
<td>flav = 1300.4 + 1263.2 × lat</td>
<td>0.0001</td>
<td>0.0797</td>
<td>0.0001</td>
<td></td>
</tr>
</tbody>
</table>

Note: *flav* is a linear term, and *tannin* is a quadratic term.
flavonoids in *Triadica* (Dyer et al., 2018; Haider et al., 2019). We also acknowledge that the current absolute invasive latitude range of *Triadica* is narrower (6 degrees), than in the native range (14 degrees).

In summary, this study is the first to examine the relationships between chemical responses to herbivory and chemical responses to climate and solar radiation in invasive plants in the context of
Our results provide novel insights into the mechanism of plant invasion, i.e., during invasion process, plants are able to adjust their secondary metabolisms to decrease secondary chemicals that primarily defend against herbivory and increase secondary chemicals that help them to respond to abiotic environments. Our findings indicate that natural enemy release (Elton, 1958) shapes plant responses to herbivory but also to abiotic environments. Our study emphasizes the importance of simultaneous consideration of plant chemical responses to abiotic and biotic environments which could help to fully understand invasive plant adaptive strategies.

Table 2

Effects of latitude, environmental variables, and herbivores on tannins (mg/g), flavonoids (mg/g), and their ratio. Chew: sqrt (% leaf area consumed); Roll: sqrt (% of leaves rolled). Suck: sqrt (abundance of sucking herbivores), avgT (°C), maxT (°C), precip (mm), Srad (kJ m$^{-2}$ day$^{-1}$). Significant effects ($P$<0.05) indicated in bold.

<table>
<thead>
<tr>
<th></th>
<th>Tannins</th>
<th>Flavonoids</th>
<th>T:F</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$F_{1,183}$</td>
<td>$P$</td>
<td>Estimate</td>
</tr>
<tr>
<td>Lat</td>
<td>1.1</td>
<td>0.3060</td>
<td>−16.5787</td>
</tr>
<tr>
<td>Lat$^2$</td>
<td>0.6</td>
<td>0.4385</td>
<td>−0.1849</td>
</tr>
<tr>
<td>avgT</td>
<td>1.0</td>
<td>0.3244</td>
<td>−5.4434</td>
</tr>
<tr>
<td>maxT</td>
<td>2.0</td>
<td>0.1604</td>
<td>−2.8907</td>
</tr>
<tr>
<td>precip</td>
<td>1.3</td>
<td>0.2573</td>
<td>−0.02705</td>
</tr>
<tr>
<td>Scad</td>
<td>0.6</td>
<td>0.4412</td>
<td>−0.00348</td>
</tr>
<tr>
<td>Chew</td>
<td>1.3</td>
<td>0.2593</td>
<td>−0.2152</td>
</tr>
<tr>
<td>Roll</td>
<td>0.3</td>
<td>0.5621</td>
<td>−0.163</td>
</tr>
<tr>
<td>Site</td>
<td>1.9</td>
<td>0.0322</td>
<td>2.1</td>
</tr>
</tbody>
</table>

Fig. 4. The dependence of foliar flavonoid and tannin concentrations on whether a tallow tree was from a population in China or the US (95% confidence interval of Hedges' $g$) together with the dependence of foliar flavonoid and tannin concentrations on latitude where the population occurred in China or the US (95% confidence interval of Fisher's $z$ back-transformed to $r$). The data are from previously published common garden studies which contain the foliar flavonoid and tannin concentrations of *Triadica* populations from China and the US.
Declaration of competing interest

All authors declare that they have no competing interests.

Acknowledgments

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Data and materials availability

All data needed to evaluate the conclusions in the paper are present in the paper and/or the Supplementary Materials. Additional data related to this paper may be requested from the authors.

Appendix A. Supplementary data

Supplemental data to this article can be found online at https://doi.org/10.1016/j.scitotev.2020.140452.

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