Invasive *Spartina alterniflora* exhibits increased resistance but decreased tolerance to a generalist insect in China

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Abstract

Several theories explaining the post-introduction evolution of invasive plants predict that specialist-enemy release often leads to decreased defense in introduced plants, but how novel generalist consumers in the introduced ranges affect plant-defense evolution remains largely unexplored. According to the shifting defense hypothesis, the decreased defense against specialist enemies may result in the increased defense against generalists. We compared resistance and tolerance of native (from the USA) and invasive populations (from China) of *Spartina alterniflora* to the dominant generalist insect *Laelia coenosa* in China. We also compared leaf traits between *Spartina* population origins. We found that *Laelia* had lower performance on invasive than on native *Spartina* populations. Native *Spartina* populations, however, had a greater capacity to compensate for leaf damage by *Laelia* than invasive ones. Although specific leaf area and leaf carbon content did not significantly vary between *Spartina* origins, invasive *Spartina* populations had lower leaf-nitrogen content than native populations. These results suggest that, following its introduction to China, *Spartina* has developed increased resistance but decreased tolerance to the generalist herbivore, which may be related to evolutionary increases in leaf carbon-to-nitrogen ratio. These findings enhance our understanding of invasive plant defense against generalist herbivores in the introduced ranges and highlight the trade-off between resistance and tolerance.

Key messages

- Herbivory pressure by native generalist insects may affect post-introduction evolution of invasive plant defense strategies.
- We compared resistance and tolerance between native and invasive populations of an invasive plant to a native generalist insect.
- The invasive plant has increased resistance but decreased tolerance to the generalist following its invasions, which is related to changes in leaf nitrogen content.
- This finding enhances our understanding of invasive plant evolution and highlights a trade-off between defense strategies.

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Introduction

Biological invasions pose a major threat to natural and managed ecosystems (Jiao et al. 2018; Scott-Brown et al. 2018). Successful invasions by introduced plants have often been attributed to their rapid evolution in the introduced ranges, including changes in growth and reproductive traits and in defense against herbivores (Oduor et al. 2011). Understanding this helps reveal the mechanisms of plant invasions and thereby improve their management strategies (Wang et al. 2011). Two fundamental hypotheses have been proposed to explain how herbivores impact plant invasion success. The enemy release hypothesis (ERH) predicts that plant invasion success results from reduced regulation by specialist enemies in the introduced ranges (Keane and Crawley 2002). The evolution of increased competitive ability (EICA) hypothesis further states that under such specialist-free conditions, introduced plants may evolve decreased defense levels but increased competitive ability (Blossey and Nötzold 1995). Both hypotheses mainly consider the effects of specialist herbivores and have been extensively studied for interactions involving specialists and introduced plants (e.g., Müller and Martens 2005; Liu and Stiling 2006; Zou et al. 2008; Wang et al. 2011; Oduor et al. 2017). A shared weakness of the ERH and EICA, however, is that they do not consider the importance of novel generalist herbivores in the introduced ranges.

Increasing evidence suggests that, despite escaping from specialist herbivores, invasive plants may be frequently attacked by generalists in their introduced ranges (reviewed by Bezemer et al. 2014). In other words, plants are often subjected to a different herbivore assemblage in their native versus introduced ranges. Therefore, studies only concerning effects of specialist enemies on invasive plants cannot provide a complete picture of the evolution of plant defense against herbivores (Huang et al. 2010). The shifting defense hypothesis (SDH) argues that if invasive plants lack their specialist enemies but are still under attack by local generalists, plants may evolve defense strategies that are less effective against specialists but more effective against generalists (Müller-Schärer et al. 2004; Joshi and Vrieling 2005; Huang et al. 2010; Orians and Ward 2010; Doorduin and Vrieling 2011). Selection pressure by generalist herbivores, therefore, may still favor allocation to defense among invasive plants, contrary to the prediction of the EICA hypothesis.

Plant defense against herbivores involves two strategies: resistance and tolerance (Strauss and Agrawal 1999). Resistance is any plant trait that reduces plant damage by herbivores or decreases fitness of herbivores, whereas tolerance is the capacity of a plant to maintain its fitness

via compensatory growth and reproduction following herbivore damage (Agrawal and Fishbein 2006). These two strategies can co-occur but are often negatively correlated in plants; an invasive plant species may have high resistance but low tolerance or vice versa (Weinig et al. 2003; Oduor et al. 2011; Wang et al. 2011). This trade-off between defense strategies is thought to occur because the amount of resources available for defense is limited (Fineblum and Rausher 1995; Leimu and Koricheva 2006). Moreover, plant defenses against herbivores are often related to leaf traits. For example, the ratio of carbon to nitrogen may affect leaf palatability to herbivores and may thus indirectly affect plant resistance (Hoffland et al. 2000; Siska et al. 2002; Moles et al. 2011). Other traits (e.g., nitrogen allocation pattern, and specific leaf area) of leaves before they are damaged can account for the capacity of plants to recover after herbivore attack and therefore may partially explain the mechanisms of plant tolerance (Fernando et al. 2008; Evans and Poorter 2013).

Because of variation in selection pressures imposed by herbivores, plant species introduced into new biogeographical ranges may reallocate defensive resources in response to new assemblages of herbivores (Oduor et al. 2011; Wang et al. 2011). Specifically, tolerance is often employed to compensate for damage caused by specialist herbivores which have adaptations that overcome the resistance of host plants (Bowers and Puttick 1988; Jokela et al. 2000; Ali and Agrawal 2012). Resistance syndromes such as chemical and physical defenses, in contrast, are predicted to vary with herbivore types with different suites of chemical defenses thought to be more or less effective against specialist versus generalist herbivores (Rosenthal and Berenbaum 1991; Muola et al. 2010). If only generalists are present in introduced ranges, they may favor genotypes of plants that have a high level of generalist resistance but a low level of tolerance (Oduor et al. 2011). This prediction is in accord with the theory of a trade-off between resistance and tolerance (Leimu and Koricheva 2006; Oduor et al. 2011). To date, however, few studies have examined this pattern of interactions involving generalist herbivores and introduced plants (but see Joshi and Vrieling 2005; Huang et al. 2010; Huang and Ding 2016).

In this study, we considered the post-introduction evolution of defense strategies (resistance and tolerance) of smooth cordgrass (*Spartina alterniflora*, hereafter '*Spartina*') in an introduced range in response to a native generalist herbivore (*Laelia coenosa*, reed tussock moth, hereafter '*Laelia*'). *Spartina* is a perennial C_4 grass, which reproduces through both seeds and clonal growth. For erosion control, soil amelioration, and dike protection, the grass was intentionally introduced to China from the USA in 1979 (An et al. 2007). After its introduction to China, *Spartina* has exhibited great invading ability (e.g., rapid growth, high

productivity, high tolerance to salinity, and well-developed root systems) and rapidly replaced native plants, making it a successful invader across the entire coast of China (Li et al. 2009). *Spartina* has been shown to experience release from specialist enemies (e.g., *Prokelisia marginata*) in its introduced range (the western coast) compared to the native range (the eastern coast) in the USA (Strong and Stiling 1983; Daehler and Strong 1997; Grevstad et al. 2003). Release from specialist herbivores has made *Spartina* evolve traits associated with rapid growth but with reduced resistance to the specialist herbivore (*P. marginata*) (Daehler and Strong 1997). Similarly, invasive *Spartina* has grown vigorously in the absence of specialist herbivores after its introduction to China. In China, however, *Spartina* is frequently attacked by the generalist herbivore *Laelia* over the past 10 years (Fig. 1).

Laelia was not reported to be found in the USA. In China, *Laelia* is a native species that is widely distributed from Heilongjiang to Guangxi (Zhao 2003), covering the whole range of *Spartina* on the eastern coast of the country (An et al. 2007). As an important insect pest, *Laelia* feeds on many other plant species, such as *Phragmites australis*, *Triarrherca sacchariflora*, *Oryza sativa*, and *Populus* spp. (Zhao 2003). Our field survey has revealed that *Laelia* feeds on *Spartina* and results in a substantial loss of leaf biomass of the plants in China (Ju et al. 2016). Although other insect species have sometimes been found on *Spartina* in China, these insects only use *Spartina* as habitats and feed less on the plants in *Spartina*-invaded salt marshes (Wu et al. 2009). As a dominant insect herbivore feeding on *Spartina* in China, *Laelia* larvae can reach a maximum density of about 30 individuals per m^2 (personal observation by R.-T. Ju). This selection pressure may lead to changes in *Spartina* defense strategies, which have not been investigated to date.

Here, we conducted laboratory and common-garden experiments to compare the resistance and tolerance of native (USA) versus invasive (China) populations of *Spartina* to *Laelia* in the introduced range. We also compared leaf traits related to plant defenses in native and invasive *Spartina* populations. Because the increased growth and reproduction of introduced *Spartina* populations compared to native *Spartina* populations has been confirmed in our previous studies (Li et al. 2009; Shang et al. 2015; Ju et al. 2017), we did not consider the increased competitive ability but focused on the defense evolution of *Spartina* in this study. Following the SDH and the hypothesized trade-off between resistance and tolerance, we made the following two predictions. First, invasive *Spartina* populations have greater resistance to but less tolerance of the generalist herbivore *Laelia* than native populations. Second, invasive *Spartina* populations have undergone evolutionary changes in leaf physiological traits which are related to the changes in defense strategies.

Materials and methods

Collection and maintenance of plants and insects

We conducted the experiments at the Shanghai Academy of Landscape Architecture Science and Planning (31°09′N, 121°26′E). In October and November 2011, we collected *Spartina* seeds in the native range from North Carolina to Texas along the southeastern coast of the USA, and from Hebei to Guangdong along the eastern coast of China (the invaded range, populations colonized in 1982). We only slightly expanded the collected populations to include the Texas Gulf Coast but did not to include the whole range of *Spartina* on the eastern coast of the USA, because we knew the invasive populations of *Spartina* in China were introduced from the southeastern coast of the USA (An et al. 2007) and sampling other populations seemed less useful. At each collection site, we randomly selected different plant individuals and then collected the seeds of each plant

Fig. 1 *Laelia coenosa* larvae feed on the leaves of *Spartina alterniflora* at the Chongming Dongtan National Nature Reserve, Shanghai, China (31°31′N, 121°58′E)

(sample size = 13–57). Each plant was about 50 m apart. All seeds from the same collection site were mixed and stored in a refrigerator at 4 °C. In August 2012, these seeds germinated in Petri dishes containing wet filter paper. These dishes were kept in an incubator at 25–28 °C. Germination was poor for three native and four invasive populations, so only three native and five invasive populations with sufficient seedlings were used for the experiments (Table 1). The germinated seedlings were maintained at 25–28 °C with a relative humidity (RH) of 80 ± 5% and a 12 h:12 h (L:D) photoperiod. When they were 2–3 cm tall, the seedlings were planted in separate trays of small pots containing vermiculite, peat, and perlite (1:1:2), and were kept in the same incubator and under the same conditions. One month later, the plants were individually transplanted into pots (25 cm diameter, 23 cm height, with one hole in the bottom) containing sediments that were collected from the Chongming Dongtan wetland (31°36′N, 121°52′E). The pots with seedlings were randomly placed in a cement pool with a 1% NaCl solution and were maintained at the same temperature, RH, and light under outdoor conditions.

Laelia caterpillars were collected from *P. australis* at the Chongming Dongtan wetland in May 2013 and were reared on fresh leaves of *P. australis* in transparent plastic jars (diameter = 8.5 cm, height = 10 cm) with 5–6 individuals per jar. The jars were placed in an incubator at 26 ± 0.5 °C with a RH of 85 ± 5% and a photoperiod of 14 h:10 h (L:D). The lid of each jar had a round hole (diameter = 3 cm) that was covered with 40-mesh (sieve size = 0.425 mm) white nylon screen. A small mass of wet cotton (dry weight = 0.5 g) was added to each jar to maintain moisture. Fresh *P. australis* leaves were supplied at 1–2 day intervals. After pupation, the pupae were maintained in the jars until they emerged as adults. The adults were then paired in new jars for oviposition, with one pair per jar, whose eggs were then moved into glass Petri dishes (diameter = 9 cm) for hatching under the same conditions as described for caterpillars rearing. Newly emerged caterpillars (< 24 h old) were used in the experiments.

Laboratory experiment (plant resistance)

We used *Laelia* performance (development, survival, leaf area consumed, and growth efficiency) to evaluate *Spartina* resistance to the generalist herbivore in a laboratory experiment conducted in August 2013. The growth efficiency was indicated by a numerical response that reflected how much growth the larva got out of leaf consumption. Excised *Spartina* leaves from each population were used in the experiment. We randomly cut the fifth–sixth youngest leaves from plants every three days and then thoroughly mixed the fresh leaves within each population for the feeding experiment. These leaves were placed in plastic valve bags and then stored in a refrigerator at 4 °C. Each newly emerged caterpillar was fed with a piece of excised leaf (randomly selected from the mixed leaves within each population). The leaf was placed on moist filter paper in a Petri dish. The Petri dish was then closed and incubated at 26–32 °C with a RH of 85 ± 5% and a 14 h:10 h (L:D) photoperiod in an incubator. To minimize error in estimating consumption, we replaced leaves with a fresh leaf daily. According to a pretest, the leaf weight per area was not significantly different among populations (one-way ANOVA, $F_{7, 79} = 0.46$, $P = 0.86$), i.e., plants of all populations had a comparable leaf mass per area. We therefore used leaf area to show larval consumption. We imaged each leaf before it was offered to a caterpillar, and again on the same day, it was removed from the Petri dish. ImageJ 2 × 2.1.4.7 software (https://imagej.nih.gov/) was used to calculate the leaf area consumed by each larva in these images. Larval survival and developmental time (duration from the first instar to the pre-pupa) were recorded. After larval pupation, each pupa together with its cocoon was weighed to the nearest 0.1 mg with a balance (BS224S, Sartorius Group, Germany) (W_g). The pupae in the cocoons were then reared under the same conditions as larvae until adult emergence. After adult emergence, each cocoon was immediately weighed (W_c) with a Sartorius BS224S balance, and the pupal net weight was then calculated ($W_g − W_c$). During the experiment, pupal survival was 100% so we only

Table 1 Native (United States) and invasive (China) populations of *Spartina alterniflora* used as seed sources in this study

Population	Site of seed collection	Abbreviation	Latitude	Longitude	Sample size
Native	United States				
	Texas Point, Texas	TP	29°42′N	− 93°51′W	57
	Mosquito Lagoon, Florida	ML	28°57′N	− 80°50′W	13
	Fort Pierce, Florida	FP	27°28′N	− 80°19′W	21
Invasive	China				
	Tanghai, Hebei	TH	39°02′N	118°20′E	37
	Binhai, Tianjin	BH	38°58′N	117°45′E	51
	Yanchen, Jiangsu	YC	33°32′N	120°38′E	29
	Chongming, Shanghai	CM	31°31′N	121°58′E	22
	Zhuhai, Guangdong	ZH	22°25′N	113°37′E	13

recorded pupal developmental time. Each treatment (leaves of eight *Spartina* populations) was replicated 15 times with one caterpillar per replicate. The growth efficiency index (GEI, expressed as mg per cm^2 leaf) for each larva was calculated as follows:

$$GEI = P/TLA \qquad (1)$$

where TLA is the total leaf area consumed by the larva, and *P* is the net weight of the pupa that developed from the larva. A larger TLA or a higher GEI value indicates lower resistance of *Spartina* to *Laelia* herbivory.

Common-garden experiment (plant tolerance)

We measured plant growth parameters in a common-garden experiment to assess *Spartina* tolerance to *Laelia*. In late July 2013, six plants of similar size (height = 30 ± 2 cm) were randomly selected from each population. All plants were placed in cages with one plant per cage. For each population, three plants were subjected to herbivory and the other three (controls) were not, i.e., each treatment was replicated three times with one plant per replicate. Two sixth-instar *Laelia* caterpillars were released on each plant assigned to the herbivory treatment. Plants together with cages were then placed in the cement pool under the same conditions as described for the maintenance of outdoor seedlings. Caterpillars were checked daily, and dead ones were replaced with living ones of the same age. After one month, when the defoliation for each plant reached about 80%, all caterpillars were removed. The plants were then allowed to regrow for 20 days before they were harvested. The number of ramets of each plant was counted. The aboveground (leaf and stem) and belowground (root) parts of plants were separated and dried at 70 °C for 48 h and then weighed. The aboveground, belowground, and total biomasses were calculated. We used the relative growth index (RGI) of ramet number and the RGI of biomass after insect herbivory to indicate the tolerance level of each population. This index was calculated as follows:

$$RGI = G_e/G_c \qquad (2)$$

where G_e is the ramet or biomass data from the herbivory treatment, and G_c is the ramet or biomass data from the no-herbivory control. A higher RGI value indicates higher tolerance (Huang et al. 2010).

Leaf traits

To compare physiologically relevant leaf traits between invasive and native *Spartina* populations, we measured the contents of carbon, nitrogen, and water, and specific leaf area for all the eight populations. In August 2013, we randomly collected 18 *Spartina* leaves per population. These were the fifth–sixth leaves from 18 individual plants within the same population. All leaves for each population were thoroughly mixed and then randomly divided into three replicates with six leaves per replicate. The leaves were placed in plastic valve bags and then immediately placed in a refrigerator at 4 °C. Leaf area (LA) was measured with an LI-3050C Area Meter (LI-COR, USA). Leaves were weighed before (W_1) and after being dried (W_2). Dried leaves were ground, and total carbon (C) and nitrogen (N) contents were analyzed with a Flash EA 1112 Elemental Analyzer (Thermo Electron Corporation, Italy), and the C: N ratio was then calculated. Percent water content (W) and specific leaf area (SLA, cm^2 per g dry mass) were calculated with the following formulas:

$$W = \left((W_1 - W_2)/W_1 \right) \times 100\% \qquad (3)$$

$$SLA = LA/W_2 \qquad (4)$$

Statistical analyses

Linear mixed-effects models (LMMs) or generalized linear mixed-effects models (GLMMs) were used to examine the differences between native and invasive populations of *Spartina* regarding resistance (in the laboratory experiment), plant tolerance (in the common-garden experiment), and leaf traits. Models included plant origin (native vs. invasive) as a fixed effect and plant population (eight collection populations) nested within origin as a random effect. Because of the strong correlation between some response variables (Pearson $r > 0.7$; Table S1, S2, S3), we avoided including more than one of these variables in the following analysis. The discarded variables included pupal weight, RGI for belowground biomass, leaf water content, and leaf C: N ratio. We validated the use of LMMs with the restricted maximum likelihood estimation method (REML) based on the normalized scores of standardized residual deviance of response variables for the continuous data of total leaf area consumed and SLA. The following continuous data were fitted to a 'gamma' family with 'log' link using the maximum likelihood (Laplace Approximation) method of GLMMs based on the normalized scores of standardized residual deviance of response variables: GEI for larva, leaf C and N contents, and RGIs for ramet number, aboveground biomass, and total biomass. The following count data were fitted to a 'Poisson' family with 'log' link using the maximum likelihood (Laplace Approximation) method of GLMMs: larval developmental time and pupal developmental time. The only binary variable (larval survival) was fitted to a 'binominal' family with 'logit' link using the maximum likelihood (Laplace Approximation) method of GLMMs. We used likelihood ratio tests to calculate the significance of the random effect. Additionally, the RGIs for plant total biomass after herbivory were regressed against resistance values (i.e., 100/total leaf area consumed). This enabled us

to test the trade-off between resistance and tolerance among the eight populations. Analyses were performed with the statistical package R 3.0.0 (using the 'lme4' package) with a significance level of alpha = 0.05 for LMMs and GLMMs (R Development Core Team 2013).

Results

Developmental times of *Laelia* larvae and pupae depended significantly on *Spartina* population nested within plant origin (Table 2). Larval developmental time, pupal developmental time, and larval survival did not depend on *Spartina* origin, i.e., no differences were observed between insects reared on plants of native versus invasive *Spartina* populations (Table 2). Total leaf area consumed by larvae and the GEI for larva, however, were significantly greater for *Laelia* feeding on leaves of native *Spartina* populations than those of invasive populations (Table 2).

The RGI for ramet number did not differ between invasive and native populations (Table 2). The RGIs for total biomass and aboveground biomass were significantly higher

(RGIs > 1) for native populations than for invasive populations (RGIs < 1) (Table 2). In addition, RGI variables were independent of population nested within origin (Table 2). These results indicated that native populations performed better (i.e., had higher RGIs) than invasive populations when challenged with *Laelia* herbivory. Moreover, there was a negative correlation between resistance and tolerance among the eight *Spartina* populations (Fig. 2).

SLA and leaf C content did not depend on plant origin, i.e., no differences were found between native and invasive populations of *Spartina* (Table 2). Leaf N content, however, was significantly higher for native than for invasive populations (Table 2). All three leaf traits differed among populations nested within origin (Table 2).

Discussion

Evolution of herbivore resistance

Although the number of geographical populations assessed was relatively small in this study, *Laelia* larvae consumed

Table 2 Mixed-nested analyses of variance for insect performance (plant resistance), plant growth (plant tolerance)[a], and leaf traits between native and invasive populations of *Spartina alterniflora*

Category	Response variable	Origin[b]			Population (origin)[b]			Native (mean ± SE)	Invasive (mean ± SE)
		d.f.	F/LRT[c]	P[d]	d.f.	F/LRT[c]	P[d]		
Insect performance (determined in a laboratory experiment)	Larval developmental time (days)	1, 6	3.59	0.058	6, 69	3.48	**0.007**	62.96 ± 1.17	68.08 ± 1.07
	Larval survival	1, 6	0.54	0.461	6, 112	0.00	0.247	0.60 ± 0.07	0.67 ± 0.06
	Pupal developmental time (days)	1, 6	0.21	0.647	6, 69	5.51	**0.002**	6.89 ± 0.12	7.18 ± 0.10
	Total leaf area consumed (cm² per larva)	1, 6	20.26	**0.003**	6, 69	0.02	0.205	319.31 ± 10.57	244.97 ± 7.42
	GEI for larva[e] (mg per cm²)	1, 6	18.88	**< 0.001**	6, 69	0.86	0.061	1.16 ± 0.04	0.94 ± 0.02
Plant growth (determined in a common-garden experiment)	RGI[f] for ramet number	1, 6	0.02	0.910	6, 16	0.00	0.305	0.97 ± 0.18	0.96 ± 0.06
	RGI for total biomass	1, 6	12.01	**0.003**	6, 16	0.00	0.302	1.24 ± 0.05	0.89 ± 0.06
	RGI for aboveground biomass	1, 6	7.47	**0.007**	6, 16	0.12	0.201	1.31 ± 0.10	0.76 ± 0.06
Leaf traits	Specific leaf area (cm² per g)	1, 6	< 0.01	0.999	6, 16	7.43	**0.002**	121.93 ± 2.30	121.93 ± 2.54
	Leaf carbon content (%)	1, 6	2.04	0.416	6, 16	16.00	**< 0.001**	42.77 ± 0.24	42.19 ± 0.21
	Leaf nitrogen content (%)	1, 6	19.18	**0.015**	6, 16	13.44	**< 0.001**	2.16 ± 0.07	1.58 ± 0.06

[a]*Spartina* resistance to generalist herbivory was assessed in a laboratory experiment, and *Spartina* tolerance of generalist herbivory was assessed in a common-garden experiment

[b]Origin: native versus invasive; population: eight populations shown in Table 1

[c]LRT is the value of the likelihood ratio test

[d]Significant results are shown in bold (P < 0.05)

[e]GEI is the growth efficiency index, which was calculated by dividing pupal weight by the leaf area consumed

[f]RGI is the relative growth index, which was calculated by dividing the data from the herbivory treatment by the data from the no-herbivory control
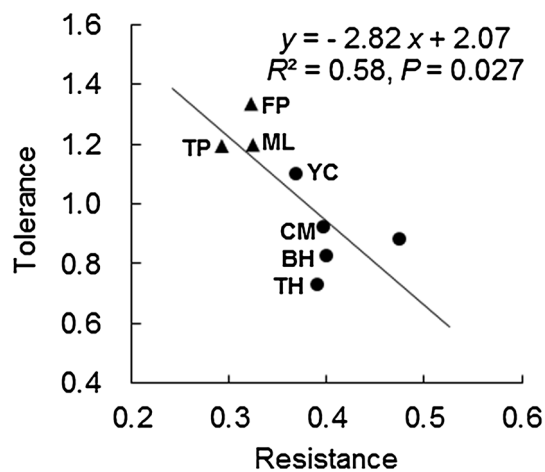
Fig. 2 Linear regression showing the trade-off (i.e., negative correlation) between resistance to and tolerance of *Laelia coenosa* herbivory in eight populations of *Spartina alterniflora*. Resistance value was calculated as 100/the total leaf area consumed per larva in each population. Tolerance was calculated as the relative growth index for total plant biomass after herbivory. Values are population means. Triangles and circles represent native populations and invasive populations, respectively. The population abbreviations are shown in Table 1

significantly less leaf area and had a significantly lower GEI when feeding on *Spartina* leaves from invasive populations compared to those from native populations (Table 2). Our past study using a choice bioassay has also confirmed that *Laelia* prefers to feed on native populations rather than introduced populations of *Spartina* (plant populations used were the same as in this study) (Ma et al. 2015). In terms of development and feeding capacity of the caterpillars, the poorer performance of *Laelia* on invasive *Spartina* populations indicates that *Spartina* resistance to *Laelia* might be higher for invasive than for native *Spartina* populations. Additionally, we have also reported that plant traits related to growth, reproduction and competition of invasive *Spartina* populations are superior to native *Spartina* populations (Li et al. 2009; Shang et al. 2015; Ju et al. 2017). The results of increased resistance to generalists and increased growth of invasive plants have also been found in some other studies (e.g., Leger and Forister 2005; Müller and Martens 2005; Ridenour et al. 2008; Caño et al. 2009). Both these earlier studies and the current findings suggest that when introduced plants encounter generalists in the new ranges, plants may simultaneously increase investment in resistance and competitiveness after their invasions. This result is inconsistent with the EICA hypothesis but is consistent with the SDH.

Plant resistance to herbivores is often associated with leaf palatability (Moles et al. 2011), which is influenced by the contents of C and N and their ratio in leaves (Hoffland et al. 2000). Nitrogen is a main component of plant amino acids and proteins, which can affect the development and reproduction of herbivores (McNeill and Southwood 1978;

Mattson 1980). In addition, if protein content is low relative to carbohydrate content in certain plants, these plants will have higher C: N ratios and their leaves will be hardened, which are associated with reduced herbivore preference and performance (Siska et al. 2002; Agrawal and Fishbein 2006; Moles et al. 2011). In our study, invasive *Spartina* populations did not differ from native populations in terms of C content but had lower N content than native populations (Table 2). The leaf C: N ratio, therefore, increased in invasive *Spartina* populations versus native populations. This change might have contributed to the decreased palatability of invasive *Spartina* to *Laelia*. Apart from C: N ratio, the novel weapon hypothesis (Enge et al. 2012) suggests that chemical defense traits such as phenolics in leaves can also mediate *Spartina* resistance to herbivores (Siska et al. 2002), more traits are therefore needed to be included in further studies.

Evolution of herbivore tolerance

Concerning the defense strategies of invasive plants against herbivores, tolerance has received less attention than resistance. In addition, few studies have simultaneously examined the resistance and tolerance of invasive plants to generalist herbivory (but see Joshi and Vrieling 2005; Huang et al. 2010; Huang and Ding 2016). Compensatory regrowth is regarded as a key character of tolerance (Juenger and Bergelson 2000). In this study, the capacity to compensate for leaf damage caused by *Laelia* was lower for invasive *Spartina* populations than for native populations (Table 2). Interestingly, invasive populations showed undercompensation (RGIs < 1), but natives exhibited overcompensation (RGIs > 1) with regard to biomass accumulation in response to leaf herbivory. This result suggests that invasive populations might have reduced their tolerance to *Laelia* herbivory in the introduced range. Juenger and Bergelson (2000) have reported that the evolution of compensation patterns has important implications for plant reproduction, and overcompensating plants generally do not optimally reproduce after herbivory damage. The reduction in compensation capacity of invasive *Spartina* populations, therefore, may be a trade-off between their reproduction and tolerance of herbivory.

Although identifying the mechanisms of tolerance is still a major challenge in plant biology (Fornoni 2011), research has confirmed that a plant's ability to exhibit compensatory photosynthesis is related to the amount of resources in leaves before herbivore damage occurs (Fernando et al. 2008; Evans and Poorter 2013). In our study, the greater capacity for compensatory growth for native than for introduced *Spartina* populations after *Laelia* herbivory might have resulted from the higher leaf N contents in the native populations (Table 2). This is because the rate of compensatory photosynthesis is facilitated by photosynthetic enzymes,

which are positively correlated with leaf N concentrations (Fernando et al. 2008). High leaf N content can contribute to the photosynthesis of the remaining leaves and thus favor plant regrowth after herbivory. Difference in leaf N content may partially explain the higher tolerance of native than invasive *Spartina* populations to *Laelia*. Because plant traits induced by herbivores may also participate in tolerance (Fornoni 2011), additional research is needed to examine the roles of plant constitutive (pre-damage) versus induced (post-damage) traits on the tolerance of native versus introduced *Spartina* populations.

Trade-offs between resistance and tolerance

Previous studies dealing with the evolution of defense strategies in invasive plants have often found a negative correlation between resistance and tolerance to herbivory. For instance, invasive populations of *Brassica nigra* have higher resistance but lower tolerance to herbivory than native populations in the introduced ranges (Oduor et al. 2011). Invasive *Triadica sebifera* (= *Sapium sebiferum*) populations, in contrast, show less resistance but greater tolerance to specialist herbivory than the native populations (Zou et al. 2008; Wang et al. 2011). Although no clear pattern has emerged, a trade-off between resistance and tolerance may explain some of this correlation (Leimu and Koricheva 2006; Huang et al. 2010; Oduor et al. 2011). In this study, we also found evidence for a trade-off between *Spartina* resistance and tolerance to the generalist *Laelia* (Fig. 2). This trade-off might be related to leaf traits in *Spartina.* As discussed earlier, if relatively low leaf N contents account for the decreased palatability and hence for the increased resistance in invasive *Spartina* populations, such low N contents could also reduce compensatory photosynthesis of plant and hence reduce the tolerance of invasive populations.

Although the trade-offs between resistance and tolerance to herbivory have frequently been reported in introduced plants, costs play an important role in trade-offs and resource investment strategies (Núñez-Farfán et al. 2007). If resistance and tolerance serve the same defense function and both have costs, we can then expect that a plant with high resistance may not require or exhibit high tolerance because of limits in the aggregate amount of defense resources (Leimu and Koricheva 2006; Hakes and Cronin 2011). Therefore, an individual plant may simultaneously have low tolerance but high resistance and vice versa, as we discussed earlier. In the current study, the increased resistance of invasive *Spartina* populations to *Laelia* herbivory may have low fitness costs such that the plants might still be able to allocate additional resources to increase growth and competitiveness, which would be more important than increasing tolerance. On the other hand, even though *Spartina* allocates a certain proportion of resources to tolerance, this tolerance

may inadequately compensate for leaf damage by *Laelia* larvae as the insect is abundant in the introduced range. In eastern China, following rapid replacement of native plants by invasive *Spartina*, *Laelia* has increasingly shifted from using native plants to using *Spartina* such that *Laelia* population density is now very high on invasive *Spartina* (Ju et al. 2016). We speculate that a high density of *Laelia* may also affect the evolution of *Spartina* tolerance in the introduced ranges, because low tolerance may correlate with high herbivory loads (Strauss and Agrawal 1999; Bossdorf et al. 2004; Zou et al. 2008).

Herbivory and defense in native versus introduced populations

During plant invasions, resource allocation between plant resistance and tolerance to herbivores may often be related to whether the major selection pressure is from specialist or generalist herbivores. Indeed, *Spartina* populations suffer from different herbivory types in their native versus introduced ranges. In the native range in North America, *Spartina* is often attacked by several species of specialist herbivores and sometimes by generalists (Strong and Stiling 1983; Stiling et al. 1991; Grevstad et al. 2003) but not by *Laelia*. In such native environments, the high selection pressure by abundant specialists might have selected for plant genotypes that have low resistance but high tolerance (Müller-Schärer et al. 2004; Joshi and Vrieling 2005). In the introduced range in China, *Spartina* has grown vigorously in specialist-free environments. As a response to release from specialist herbivores, tolerance of *Spartina* might have become reduced in the introduced range. Given that *Laelia* has become a dominant herbivore on *Spartina*, there may have been rapid evolution of *Spartina* resistance in China. It supports the idea that herbivore communities dominated by generalists in the introduced ranges exert a strong directional selection pressure so that introduced plants can rapidly evolve high resistance but low tolerance to generalists (Weinig et al. 2003; Oduor et al. 2011). In addition to herbivore pressure, it is likely that abiotic factors (e.g., soil, temperature and precipitation) in introduced ranges also contribute to the defense evolution of introduced plants, but these have not yet been studied.

In this study, most differences in resistance and tolerance as well as associated leaf traits can be attributed to differences in *Spartina* origin (invasive vs. native). Within the same origin, however, some parameters (e.g., larval and pupal developmental times, and leaf C and N contents) exhibited significant variability among populations (Table 2). This suggests that the defense traits of *Spartina* may differ among latitudinal clines within invasive or native ranges. Previous studies have documented that latitudinal variation in herbivory plays an important role in mediating

the distribution patterns of *Spartina* in North America (Pennings et al. 2001, 2009; Salgado and Pennings 2005). Similarly, the growth and reproductive traits of *Spartina* vary considerably across latitudes on the eastern coast of China (Liu et al. 2016, 2017). Perhaps, can only the investigation of additional populations reveal whether or not the post-introduction evolution of *Spartina* defense strategies differs along a latitudinal gradient in the introduced ranges. This possibility needs to be considered in further biogeographical studies.

Conclusions

The results of this study show that, relative to its native populations from the USA, invasive *Spartina* populations from China have evolved to higher resistance but lower tolerance to the dominant generalist herbivore *Laelia* in the introduced ranges. The results also indicate that the N content and C: N ratio in leaf tissues may play key roles in the defense evolution of introduced *Spartina* after its invasion. Such findings highlight a trade-off between plant resistance and tolerance to generalist herbivory among *Spartina* populations of different origins. However, the number of populations considered here was rather small, and a fuller understanding of the evolution of defense by *Spartina* may require the study of a greater number of populations from both native and introduced ranges. Future research is also required to consider different types of herbivores in multiple invaded ranges and a broader set of plant traits (e.g., secondary metabolites) relevant to *Spartina* defense strategies.

Author contributions

RTJ, DM and BL designed and performed the experiments. RTJ, DM, ES and XL analyzed the data. RTJ, DM, ES, JHW and BL wrote the manuscript. All authors read and approved the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This manuscript does not contain any studies involving human participants and/or animals.

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