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Evolutionary increases in defense during a biological invasion

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Abstract Invasive plants generally escape from specialist herbivores of their native ranges but may experience serious damage from generalists. As a result, invasive plants may evolve increased resistance to generalists and tolerance to damage. To test these hypotheses, we carried out a common garden experiment comparing 15 invasive populations with 13 native populations of *Chromolaena odorata*, including putative source populations identified with molecular methods and binary choice feeding experiments using three generalist herbivores. Plants from invasive populations of *C. odorata* had both higher resistance to three generalists and higher tolerance to simulated herbivory (shoot removal) than plants from native populations. The higher resistance of plants from invasive populations was associated with higher leaf C content and densities of leaf trichomes and glandular scales, and lower leaf N and water contents. Growth costs were detected for tolerance but not for resistance, and plants from invasive populations of *C. odorata* showed lower growth costs of tolerance. Our results suggest that invasive plants may evolve to increase both resistance to generalists and tolerance to damage in introduced ranges, especially when the defense traits have low or no fitness costs. Greater defenses in invasive populations may facilitate invasion by *C. odorata* by reducing generalist impacts and increasing compensatory growth after damage has occurred.

Keywords Costs · Evolution of increased competitive ability hypothesis · Generalist herbivore · Resistance · Tolerance

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Introduction

Post-introduction evolution is often considered as an important mechanism underlying invasion success of introduced plant species (Blossey and Nötzold 1995; Müller-Schärer et al. 2004; Joshi and Vrieling 2005; Feng et al. 2009; Oduor et al. 2011). The evolution of increased competitive ability (EICA) hypothesis predicts that after liberation from herbivores, invasive plants evolve increased vigor and decreased defense by reallocating resources from costly defensive traits to growth or reproduction (Blossey and Nötzold 1995). Evidence for increased vigor (Flory et al. 2011; Alba and Hufbauer 2012), decreased defense (Maron et al. 2004; Wolfe et al. 2004), or both predictions of the EICA hypothesis (Siemann and Rogers 2003; Feng et al. 2009; Fukano and Yahara 2012) has been documented in some invasive species. However, inconsistent or even contrary results are also found in other invasive species (Leger and Forister 2005; Müller and Martens 2005; Caño et al. 2009; Cripps et al. 2009; Alba et al. 2011; Oduor et al. 2011; Parker et al. 2013). For example, plants from

invasive and native populations of *Lepidium draba* are not significantly different in growth, and plants from invasive populations produce more glucosinolates (Müller and Martens 2005). Similarly, Oduor et al. (2011) found that *Brassica nigra* evolved increased resistance to herbivores after introduction. Potential reasons for these inconsistent results include (1) few studies testing the EICA hypothesis have compared invasive populations with their specific source populations (but see Qin et al. 2013; Uesugi and Kessler 2013), and (2) few studies have distinguished the effects of specialist and generalist herbivores on the evolution of invasive plants (Müller-Schärer et al. 2004).

To address concerns about EICA, Müller-Schärer et al. (2004) and Joshi and Vrieling (2005) advanced the shifting defense hypothesis (SDH), which predicts that invasive plants may evolve to decrease defense against specialist herbivores but maintain or even increase defense against generalist herbivores in introduced ranges. This hypothesis is consistent with the fact that invasive plants may not escape from generalists (Müller-Schärer et al. 2004; Joshi and Vrieling 2005; Oduor et al. 2011) and may even experience increased damage in introduced ranges (Cripps et al. 2009), although they generally are released from specialists.

Plants usually synthesize toxic chemicals to defend against generalists (qualitative defense). In contrast, specialists often use the toxic chemicals to locate their host plants. To balance the harmful impacts of specialists and generalists, genotypes with intermediate levels of toxic chemicals may be selected in native ranges (Müller-Schärer et al. 2004; Joshi and Vrieling 2005). In introduced ranges, however, genotypes with high levels of toxic chemicals may be selected, which are effective in defending against generalists and do not attract specialists due to their absence. Studies testing the SDH by comparing the differences in defense against generalists between plants from invasive and native ranges have found inconsistent results (Joshi and Vrieling 2005; Leger and Forister 2005; Abhilasha and Joshi 2009; Caño et al. 2009; Cripps et al. 2009), although few studies considered the costs of defensive traits (Alba et al. 2011; Oduor et al. 2011).

Tolerance is a strategy used by plants to deal with damage due to enemies and other causes (Simms and Triplett 1994; Strauss and Agrawal 1999; Müller-Schärer et al. 2004). Tolerance allows plants to maintain fitness after damage has occurred, while resistance reduces the extent of damage (Simms and Triplett 1994; Strauss and Agrawal 1999; Müller-Schärer et al. 2004). Like resistance (Fine et al. 2006), tolerance can also evolve in response to selective pressure such as herbivory, physical disturbance or stressful environments (Belsky et al. 1993; Fornoni 2011), and may also have fitness costs (Simms and Triplett 1994; Agrawal et al. 1999). Relatively few studies testing the EICA hypothesis have considered tolerance (Bossdorf et al. 2004; Li et al. 2012).

Chromolaena odorata (L.) R. M. King & H. Robinson (Asteraceae) is a herb or subshrub native to the Americas from southern USA to northern Argentina, and is considered one of the worst terrestrial invasive plants in the humid (sub)tropics of the Old World (Kriticos et al. 2005). It harbors more than 200 natural enemies in native ranges and a quarter of them are specialists (McFadyen 1988; Zhang and Feng 2007). In China, no specialists are found for *C. odorata*, although a few specialists were introduced into Indonesia and India as biological control agents (Zhang and Feng 2007). Some generalist herbivores are documented for *C. odorata* in introduced ranges (Kluge and Caldwell 1992; Xu et al. 2011). The invader often forms dense monocultures in habitats with high levels of disturbance. It can resprout rapidly from remaining green stems, root collars, and roots after severe disturbance such as cutting or fire (te Beest et al. 2012). In introduced ranges, *C. odorata* may have (1) evolved increased resistance to generalists in response to the novel enemy regime (presence of generalists but absence of specialists), and (2) maintained or even increased tolerance to damage due to generalists and other causes such as mowing. To test these hypotheses, we compared defenses of 15 invasive populations with 13 native populations, including the putative source populations for the invasion, by using a common garden experiment and binary choice feeding experiments using three generalist herbivores in the invasive range of *C. odorata*. We also considered growth costs of resistance to generalists and tolerance to simulated herbivory (shoot removal).

Materials and methods

Study site

The common garden experiment was carried out in Xishuangbanna Tropical Botanical Garden (21°56′N, 101°15′E, 600 m a.s.l.), which is located in the southern part of Yunnan Province, southwest China. The garden covers an area of 1,125 hm^2, including ≈250 hm^2 of remnant primary tropical rainforest, and currently conserves over 12,000 plant species from the tropics and subtropics in both China and abroad. Here the mean annual temperature is 21.7 °C, with a mean of 25.3 °C in the hottest month (July) and 15.6 °C in the coolest month (January). The mean annual precipitation is 1,557 mm with a dry period from November to April. In the 0- to 20-cm soil layer of the common garden the pH was 6.34; there was 21.62 g organic matter kg^{-1}, 1.22 g total N kg^{-1}, 0.95 g total P kg^{-1}, 8.92 g total K kg^{-1}, 108 mg available N kg^{-1}, 107 mg available P

kg^{-1}, 204 mg available K kg^{-1}; the cation exchange capacity was 1.13 mmol kg^{-1}.

Materials and treatments

Seeds of *C. odorata* were collected in 2009 from 15 populations in its invasive ranges and 13 populations in its native ranges (Table S1). Within each population, seeds were collected from ten to 15 plants chosen randomly, and mixed together.

On 10 June 2010, seeds of the 28 populations were sown separately in seedling trays, which were filled with sand and forest topsoils (1:1). The seedling trays were placed in a shade house with 50 % irradiance. On 14 August 2010, when the seedlings were ≈10 cm tall, seedlings were transplanted into the common garden. Weeds were cleared before transplantation. Ten blocks, two plots per block, were established. One individual from each population was randomly assigned to each plot. The distance between neighboring seedlings was 80 cm. Seedlings were grown under 50 % irradiance and watered every morning with 600 ml per seedling in the first 2 weeks after transplantation. Afterward, seedlings were grown under natural environmental conditions and no supplemental water was added. Weeds were pulled out carefully when necessary. No pesticides were used during the experiment.

To assess the differences in tolerance to simulated herbivory (shoot removal) between *C. odorata* plants from invasive and native populations, aboveground parts of all plants in one plot of each of the ten blocks were removed at ground level on 9 January 2011.

Resistance to generalists

To evaluate the differences in leaf palatability (negatively associated with defensive ability) between *C. odorata* plants from invasive and native populations, binary choice feeding experiments were conducted using three generalist herbivores in August 2011. Larvae of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) and *Prodenia litura* (Fabricius) (Lepidoptera: Noctuidae) were purchased from the institute of Zoology, Chinese Academy of Sciences. The third-instar larvae of the two generalists were used. *Ganesella saurivonga* (Bavay & Dautzenberg) (Gastropoda: Camaenidae) was captured in nearby fields; the snail occasionally damages leaves of *C. odorata* in China. Two leaf discs (1.78 cm^2) from each invasive population (collected from plants grown in the plot without simulated herbivory) and two leaf discs from each native population were arranged (14 × 13 combinations) alternately in a circle in a 12-cm petri dish with two layers of wet filter paper. One invasive population (Jingdong, China; Table S1) was not used here because it did not have enough leaves for the choice feeding experiments. A larva or snail was put in the center of each petri dish. Larvae were starved for 4 h before experiments and choice feeding experiments lasted for 12 h. Snails were starved for 10 h before experiments and choice feeding experiments lasted for 24 h. Larvae and snails were weighed before feeding experiments. At the end of the feeding experiment, the remaining area of leaf discs was measured using a Li-3000C Portable Area Meter (LI-COR, Lincoln, NE), and the leaf area consumed by the larva or snail was calculated.

Leaf traits related to defense

Leaf traits related to defense were measured on recently mature leaves of plants grown in plots without shoot removal in August 2011. Leaf trichome densities on both surfaces and glandular scale density on the lower surface were measured on five leaves per plant and six plants per population using a stereoscope (Leica S8 APO; Leica, Wetzlar, Germany). Leaf water content was measured using a drying method (at 60 °C for 48 h) on six plants per population. Leaf C and N contents were measured on five plants per population using a Vario MAX CN Element Analyzer (Elementar Analysensysteme, Hanau, Germany).

Tolerance to simulated herbivory

On 15 November 2011, aboveground parts of all plants were harvested, dried to constant mass, and weighed. Before harvest, plant height and total branch number (lateral branches longer than 10 cm) were measured. Tolerance index was calculated as the percent change in performance (aboveground mass, height, and total branch number) caused by the simulated herbivory, i.e. $(P_{herbivory} - P_{control})/P_{control}$, where $P_{herbivory}$ and $P_{control}$ are performance of the damaged and undamaged plants from each block, respectively.

Statistical analyses

We first compared general differences in resistance and tolerance between *C. odorata* plants from 15 invasive populations and 13 native populations. In order to eliminate founder effects, we further compared the invasive populations with their potential source populations (Dlugosch and Parker 2008; Qin et al. 2013). *C. odorata* invading Asia may originate from Trinidad and Tobago based on internal transcribed spacer (ITS) sequences (Scott et al. 1998; Von Senger et al. 2002). Using nuclear (ITS) and chloroplast (*psb*A-*trn*H and *atp*B-*rbc*L) DNA sequences and 17 morphological and phenological traits, X.-Q. Yu et al. and Z.-Y. Liao et al. also found that *C. odorata* in Asia may originate from Florida and Trinidad (unpublished data). There

are shared haplotypes between the 15 invasive populations and the six native populations from USA and Trinidad and Tobago.

Effects of range, shoot removal, and their interaction on growth traits were tested using two-way nested ANOVA. Range, shoot removal, and their interaction were used as fixed factors; population nested within range, the interaction between population nested within range and shoot removal, and block were used as random factors. A significant interaction between range or population nested within range and shoot removal indicates significant differences in tolerance between ranges or populations. Differences in tolerance to shoot removal, resistance to the generalists, and leaf traits between *C. odorata* plants from invasive and native populations were analyzed using one-way nested ANOVA. Range was used as a fixed factor; population nested within range and block were used as random factors. Larva or snail mass was used as a covariate when testing the difference in resistance.

To determine whether the leaf traits measured in this study were associated with resistance, Pearson correlations between leaf palatability (amount of leaf area removed in feeding trials) and the leaf traits were carried out. To determine whether the leaf traits contributing to resistance had growth costs, linear regressions between growth traits and the leaf traits were carried out. Due to significant correlations between the leaf traits, factor analysis was used to eliminate multicollinearity problems in the regressions. Two factors (factor 1 trichome density, glandular scale density, leaf C content, and leaf water content; factor 2 leaf N content) were obtained and used in the above linear regressions. To determine whether tolerance had growth costs, linear regressions between growth traits and tolerance index were carried out. A significant negative correlation indicates growth costs (Strauss and Agrawal 1999; Bossdorf et al. 2004).

All analyses were carried out using SAS version 8 (SAS, Cary, NC). Data were transformed to meet the requirements of ANOVA (normal distribution and homogeneity of variances) when necessary. Type III sum of squares was used for unbalanced data samples in the nested ANOVA.

Results

Resistance and its growth costs

Plants from invasive populations of *Chromolaena odorata* had higher resistance to the three generalist herbivores than plants from native populations. In binary choice feeding experiments, *Helicoverpa armigera* (range, $F_{1,25} = 42.69$, $P < 0.001$; population, $F_{25,337} = 15.92$, $P < 0.001$), *Prodenia litura* (range, $F_{1,25} = 14.77$, $P < 0.001$; population, $F_{25,337} = 14.45$, $P < 0.001$), and *Ganesella saurivonga* (range, $F_{1,25} = 55.65$, $P < 0.001$; population, $F_{25,337} = 4.61$, $P < 0.001$) consumed respectively 101.9, 50.7, and 122.4 % more leaf area from native populations than from invasive populations of *C. odorata* (Fig. 1). Compared with plants from native populations, plants from invasive populations had significantly higher leaf C content (range, $F_{1,26} = 13.65$, $P = 0.001$; population, $F_{26,104} = 9.62$, $P < 0.001$), glandular scale density (range, $F_{1,26} = 38.71$, $P < 0.001$; population, $F_{26,130} = 19.32$, $P < 0.001$), and trichome densities on the upper (range, $F_{1,26} = 32.86$, $P < 0.001$; population, $F_{26,130} = 15.71$, $P < 0.001$) and lower (range, $F_{1,26} = 9.90$, $P = 0.004$; population, $F_{26,130} = 19.29$, $P < 0.001$) leaf surfaces, but lower leaf water (range, $F_{1,26} = 20.82$, $P < 0.001$; population, $F_{26,130} = 5.72$, $P < 0.001$) and

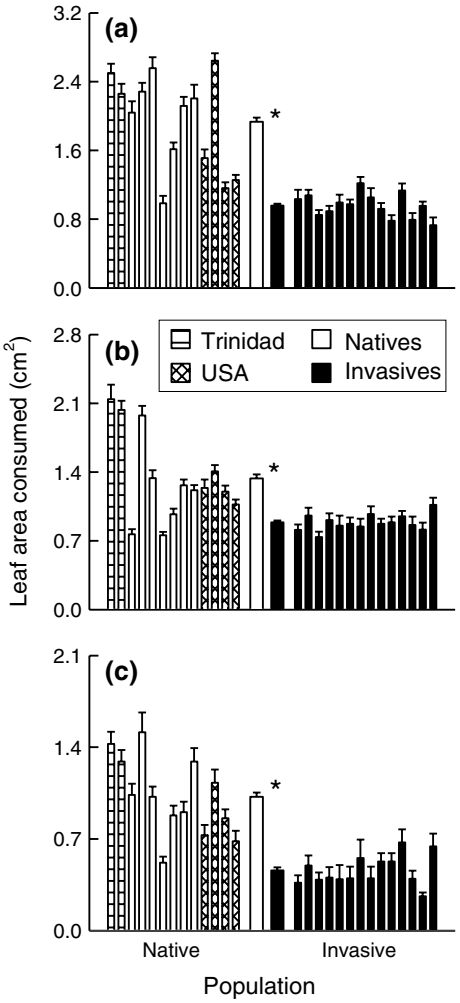

Fig. 1 Leaf area of *Chromolaena odorata* plants from native and invasive populations consumed by three different generalist herbivores: **a** *Helicoverpa armigera*, **b** *Prodenia litura*, and **c** *Ganesella saurivonga*. *Narrow bars* depict means (+SE) for populations from the native and invaded sites; *wide bars* are composite means (+SE) for each range with populations as replicates. *Asterisk* indicates significant difference ($P < 0.05$) between ranges according to one-way nested ANOVA

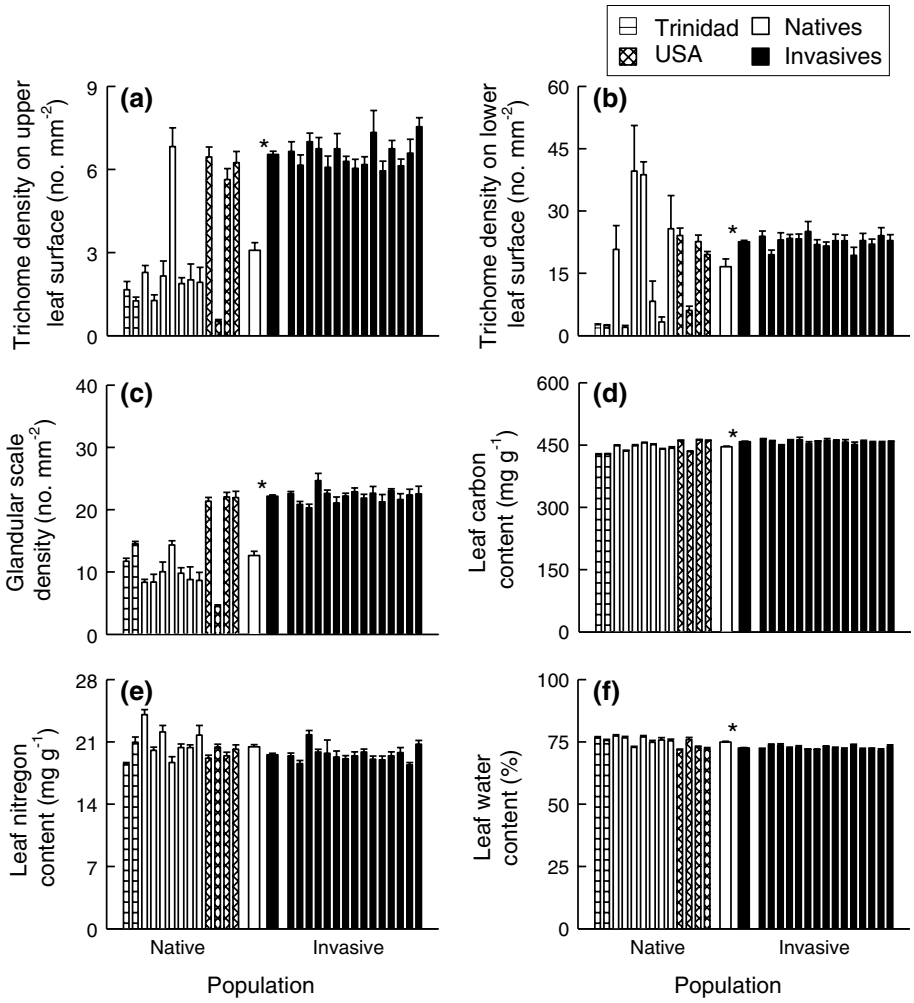

Fig. 2 Leaf traits of *C. odorata* plants from native and invasive populations related to defense. *Narrow bars* depict means (+SE) for populations from the native and invaded sites; *wide bars* are composite means (+SE) for each range with populations as replicates. *Asterisk* indicates significant difference ($P < 0.05$) between ranges according to one-way nested ANOVA

N (range, $F_{1,26} = 3.90$, $P = 0.059$; population, $F_{26,104} = 4.66$, $P < 0.001$) contents (Fig. 2).

Two main factors were obtained from the factor analysis. The first factor was related to increased trichome densities on both upper and lower leaf surfaces, glandular scale density, leaf C content, and decreased leaf water content, which explained 57.0 % of the variability in the above six leaf traits among populations (Fig. S1). The second factor was related to increased leaf N content, which explained 28.2 % of the variability among populations. Leaf area consumed by each generalist was negatively correlated with the first factor and positively correlated with the second factor (not significant for *P. litura*) for the pooled data from *C. odorata* plants of invasive and native populations (Table 1). For plants from native populations, leaf area consumed by each generalist was also negatively correlated with the first factor. No significant correlations between leaf area consumed and leaf traits were detected for plants from invasive populations. The results indicated that the densities of leaf trichomes and

Table 1 Pearson correlations between leaf area consumed by three generalist herbivores and the first two factors from factor analysis of six leaf traits for plants from invasive and native populations of *Chromolaena odorata*

	Factor 1		Factor 2	
	r	*P*	*r*	*P*
Native and invasive ($n = 27$)				
Helicoverpa armigera	−0.757	<0.001	0.502	0.008
Prodenia litura	−0.787	<0.001	−0.004	0.985
Ganesella saurivonga	−0.788	<0.001	0.414	0.032
Native ($n = 13$)				
H. armigera	−0.631	0.021	0.255	0.401
P. litura	−0.701	0.008	−0.465	0.110
G. saurivonga	−0.724	0.005	0.20	0.947
Invasive ($n = 14$)				
H. armigera	0.019	0.947	−0.316	0.271
P. litura	−0.073	0.804	0.370	0.193
G. saurivonga	−0.067	0.820	0.250	0.388

glandular scales and leaf C content were positively associated with resistance but leaf N and water contents were negatively associated with resistance of *C. odorata*.

No significant growth costs were found for the leaf traits related to resistance in *C. odorata* plants. Negative relationships were not detected between plant height (except with the second factor for pooled data), total branch number, aboveground biomass and the first two factors from factor analysis of the six leaf traits (Table S2).

Tolerance and its growth costs

The impacts of shoot removal on growth depended on plant origins (significant interaction between range and shoot removal; Table S3). Plants from invasive *C. odorata* populations had stronger tolerance based on plant height (range, $F_{1,26} = 10.46$, $P = 0.003$; population, $F_{26,217} = 3.07$, $P < 0.001$), total branch number (range, $F_{1,26} = 9.25$, $P = 0.005$; population, $F_{26,216} = 3.12$, $P < 0.001$), and aboveground mass (range, $F_{1,26} = 17.74$, $P < 0.001$; population, $F_{26,212} = 3.16$, $P < 0.001$) than plants from native populations. In general, *C. odorata* showed a strong tolerance to simulated herbivory; averaged across all populations, shoot removal did not significantly affect growth traits (Table S3). Furthermore, shoot removal stimulated growth in total branch number and aboveground mass for plants from invasive populations (Fig. 3b, c), indicating overcompensation.

The relationship between growth traits (total branch number and aboveground biomass) and tolerance index was significantly negative for plants from both invasive and native populations of *C. odorata,* indicating growth costs

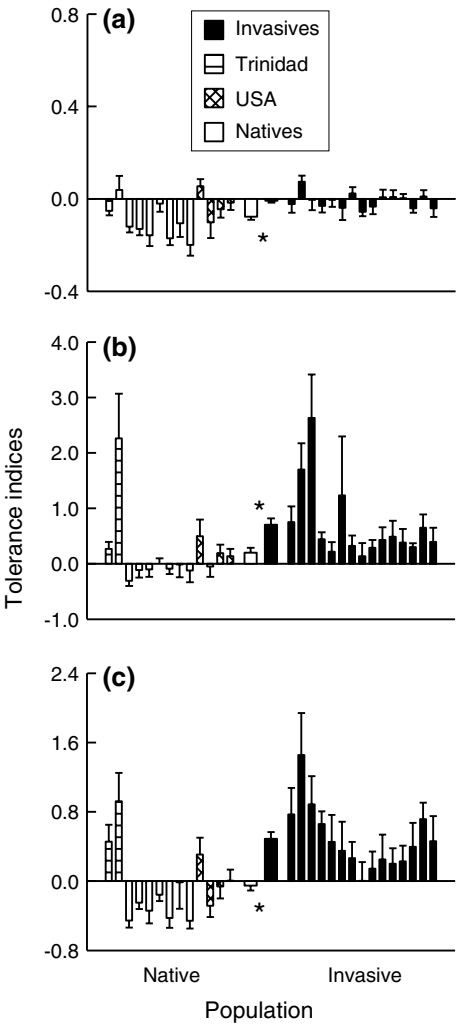

Fig. 3 Tolerance indices of *C. odorata* plants from native and invasive populations based on **a** plant height, **b** total branch number, and **c** aboveground biomass. *Narrow bars* depict means (+SE) for populations from the native and invaded sites; *wide bars* are composite means (+SE) for each range with populations as replicates. *Asterisk* indicates significant difference ($P < 0.05$) between ranges according to one-way nested ANOVA

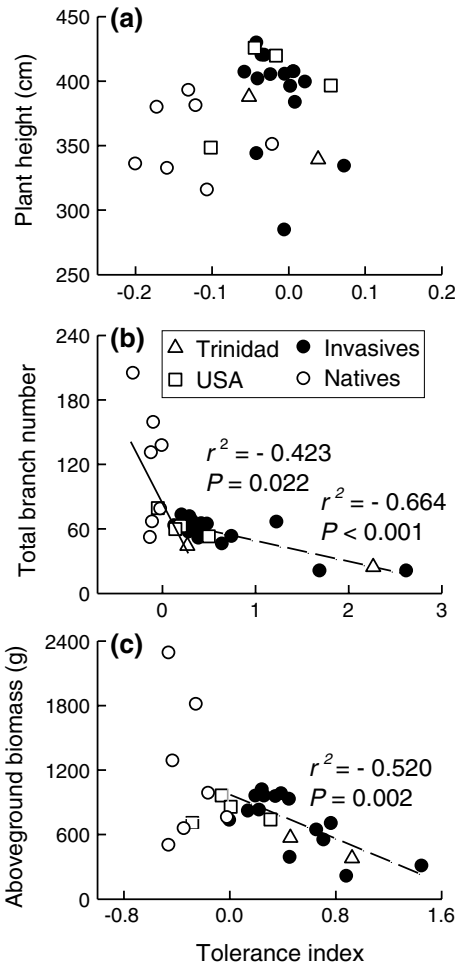

Fig. 4 Relationships between tolerance indices and **a** plant height, **b** total branch number, and **c** aboveground biomass of *C. odorata* plants from native and invasive populations grown in plots without shoot removal

of tolerance (Fig. 4b, c). Interestingly, the costs of tolerance were lower in plants from invasive populations than in plants from native populations; the slope of the regression between branch number and tolerance was much smaller in plants from invasive populations.

Comparisons between invasive populations and their potential source populations

Similar to the results from general comparisons between ranges, *C. odorata* plants from 14 invasive populations were also higher in resistance to three generalists than plants from their potential source populations (USA and Trinidad and Tobago). In binary choice feeding experiments, *H. armigera*, *P. litura*, and *G. saurivonga* consumed respectively 92.9, 87.7, and 121.7 % more leaf area from the six putative source populations than from 14 invasive populations of *C. odorata* (Table 2; Fig. 1). Compared with plants from potential source populations, plants from 15 invasive populations had significantly higher leaf C content, glandular scale density, and trichome densities on the upper and lower leaf surfaces, lower leaf water content, and similar leaf N content (Table 2; Fig. 2).

Unlike the results from general comparisons between ranges, tolerance was not significantly different between 15 invasive populations and their potential source populations (Table 2; Fig. 3).

Discussion

We found that *C. odorata* plants from invasive populations have higher resistance to generalist herbivores, resistance traits, and tolerance to simulated damage than plants from native populations. Some of these differences can be attributed to post-introduction evolution because the source populations of the invader are known.

Resistance and its growth costs

Our result that invasive populations of *C. odorata* were more resistant to generalists than native populations is contrary to the EICA hypothesis but consistent with the SDH. A similar result was also found in other studies (Joshi and Vrieling 2005; Leger and Forister 2005; Caño et al. 2009; Oduor et al. 2011). Invasive plants may not escape from generalists in introduced ranges (Müller-Schärer et al. 2004), and thus may maintain (Bossdorf et al. 2004; Huang et al. 2010) or even enhance (Joshi and Vrieling 2005; Leger and Forister 2005; Oduor et al. 2011) resistance against generalists, especially when the resistance incurs no or low costs (Müller-Schärer et al. 2004; Joshi and Vrieling 2005) as is the case for *C. odorata*.

Our results indicated that both structural and chemical defensive traits contributed to the stronger resistance of *C. odorata* plants from invasive populations compared with plants from native populations. Leaf trichome density is often considered to be a physical defensive trait to herbivores (Yamawo and Hada 2010). Glandular scales are associated with synthesis and release of secondary metabolism compounds and are often considered as an indicator of chemical defense (Yamawo and Hada 2010). Higher levels of 16 leaf defensive compounds were indeed found for plants from invasive populations compared with plants from native populations (L.-K. Zhang et al., unpublished data). Leaf C, N, and water contents are associated with leaf palatability (Agrawal et al. 2005) and also differed between native and invasive populations.

Consistent with our result, fitness costs were also not documented for traits defending against generalists in other invasive plants. For example, plants from invasive populations have more defensive chemicals and higher fitness than plants from native populations in *Senecio jacobaea* (Joshi and Vrieling 2005) and *Brassica nigra* (including higher leaf sinigrin concentration and trichome density; Oduor et al. 2011). Alba et al. (2011) found that higher fitness of *Verbascum thapsus* is also not associated with lower chemical defense (iridoid glycosides) or structural defense (leaf trichomes and toughness). However, we could not exclude the possibility that we failed to detect the costs of the defensive traits. High variation in resource acquisition among plants from different populations may make the costs of resistance undetectable (Alba et al. 2011). The costs of resistance may also be undetectable if far fewer resources are allocated to resistance traits than to other traits (Simms and Triplett 1994). In addition, costs are difficult to detect for resistance traits that perform more than one function (Siemens et al. 2002). This is the case for *C. odorata*; many chemicals of the invader have the ability to both defend against generalists and inhibit competitors (L.-K. Zhang et al., unpublished data).

Tolerance and its growth costs

We found that *C. odorata* had extremely strong tolerance to simulated herbivory and that the tolerance was stronger in plants from invasive populations than in plants from native populations. The strong tolerance may contribute to invasiveness of the invader but decrease the effectiveness of leaf-feeding biocontrol agents (Li et al. 2012). Increased tolerance was also found for other invasive plants compared with their native conspecifics (Zou et al. 2008; Abhilasha and Joshi 2009; Wang et al. 2011). Besides herbivory, tolerance is also influenced by damage due to other causes (Belsky et al. 1993; Müller-Schärer et al. 2004; Li et al. 2012). For example, Lennartsson et al. (1997) found

Table 2 Resistance to generalist herbivores, putative resistance traits, and tolerance to simulated herbivory among populations of *C. odorata* from potential source ($n = 6$, specifically Trinidad and USA) and invaded sites ($n = 14$ for resistance; $n = 15$ for others)

Variables	Native	Invaded	Range			Population (range)		
			MS	*F*-value	*P*-value	MS	*F*-value	*P*-value
Resistance to generalists (leaf area consumed by each generalist; cm^2)								
Helicoverpa armigera	1.89 ± 0.08	0.98 ± 0.03	5.92	23.55	<0.001	0.25	11.99	<0.001
Prodenia litura	1.52 ± 0.06	0.81 ± 003	4.44	35.23	<0.001	0.13	6.05	<0.001
Ganesella saurivonga	1.02 ± 0.05	0.46 ± 0.02	5.69	36.91	<0.001	0.15	3.49	<0.001
Traits related to resistance								
Leaf C content (mg g^{-1})	445.43 ± 2.97	457.93 ± 0.92	3,348.21	7.47	0.013	448.15	9.72	<0.001
Leaf glandular scale density (no. mm^2)	16.02 ± 1.13	22.17 ± 0.22	972.58	11.39	0.003	85.35	23.35	<0.001
Trichome density on lower leaf surface (no. mm^2)	12.90 ± 1.64	22.57 ± 0.39	2,405.48	13.57	0.002	177.31	21.41	<0.001
Trichome density on upper leaf surface (no. mm^2)	3.63 ± 0.44	6.55 ± 0.11	218.77	16.75	<0.001	13.06	15.10	<0.001
Leaf N content (mg g^{-1})	19.77 ± 0.22	19.56 ± 0.16	0.95	0.26	0.617	3.65	2.50	0.003
Leaf water content (%)	74.11 ± 0.39	72.51 ± 0.15	65.83	6.99	0.016	9.42	5.37	<0.001
Tolerance index based on height, branch, and biomass								
Plant height	−0.01 ± 0.01	−0.01 ± 0.01	0.01	0.85	0.369	0.01	1.40	0.133
Total branch number	0.58 ± 0.18	0.71 ± 0.11	0.10	0.66	0.426	0.15	2.61	<0.001
Aboveground biomass	0.25 ± 0.09	0.49 ± 0.08	0.30	3.04	0.098	0.10	1.85	0.022

Resistance to herbivory was measured by leaf area consumed in choice feeding experiment; for comparison of native and invaded values are mean ± SE. Results of one-way nested ANOVA are reported for each species and trait ($n = 168$ for resistance; $n = 105$ for leaf C and N contents, $n = 126$ for other leaf traits related to resistance; $n = 189$ for tolerance). Range was treated as a fixed factor; population nested within range was treated as a random factor

that grazing and mowing select for genotypes with high tolerance in *Gentianella campestris*. Several reasons may explain the higher tolerance of *C. odorata* plants from invasive populations compared with plants from native populations. First, mechanical damage such as cutting and trampling are common for *C. odorata* in introduced ranges. It generally invades habitats with strong human activity, for example disturbed forests, plantations, pastures, croplands, waste land, roadsides, riverbanks, and fallow fields (Koutika and Rainey 2010). Second, apical parts of 80.9 % of the branches of *C. odorata* plants from invasive populations die of desiccation or frost in the dry season in our common garden. Third, farmers often burn this plant, also causing death of apical meristems. Finally, *C. odorata* does not completely escape from generalists in introduced ranges; *Orthezia quadrua* (Xu et al. 2011), snails, and aphids (personal observation) occasionally damage young leaves and stems of *C. odorata* in fields. These factors can exert enough selective pressure on invasive plant species, helping *C. odorata* to enhance its tolerance in invasive ranges.

Consistent with our results, costs of tolerance were also found in other invasive plants (Zou et al. 2008; Oduor et al. 2011; Wang et al. 2011). However, tolerance with low or no costs was also found in *Gentianella campestris* (Lennartsson et al. 1997), *Raphanus raphanistrum* (Agrawal et al. 1999), and *Alliaria petiolata* (Bossdorf et al. 2004). In these studies, tolerance costs were determined by analyzing the correlation between the fitness of damaged plants and the fitness of undamaged plants. Using this method, high variance in fitness across populations may obscure potential costs of tolerance (Strauss and Agrawal 1999). To the best of our knowledge, no effort has been made to compare the difference in costs of tolerance between plants from invasive and native populations of introduced plants (but see Bossdorf et al. 2004). We do not know the reasons for the lower costs of tolerance in *C. odorata* plants from invasive populations compared with plants from native populations; but the decreased costs may be helpful for the invader.

Conclusion

Plants from invasive populations of *C. odorata* showed both higher resistance to generalist herbivores and higher tolerance than plants from native populations. Because invasive populations also had higher resistance than the putative source populations that had been identified with molecular methods, these differences are likely due to post-introduction evolution. Our results indicate that invasive plants may evolve to increase both resistance to generalists and potential tolerance to damage in introduced ranges, especially when the defense traits have low or no fitness costs.

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