

# Integrating novel chemical weapons and evolutionarily increased competitive ability in success of a tropical invader

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### Summary

• The evolution of increased competitive ability (EICA) hypothesis and the novel weapons hypothesis (NWH) are two non-mutually exclusive mechanisms for exotic plant invasions, but few studies have simultaneously tested these hypotheses. Here we aimed to integrate them in the context of *Chromolaena odorata* invasion.

• We conducted two common garden experiments in order to test the EICA hypothesis, and two laboratory experiments in order to test the NWH.

• In common conditions, *C. odorata* plants from the nonnative range were better competitors but not larger than plants from the native range, either with or without the experimental manipulation of consumers. *Chromolaena odorata* plants from the nonnative range were more poorly defended against aboveground herbivores but better defended against soilborne enemies. *Chromolaena odorata* plants from the nonnative range produced more odoratin (*Eupatorium*) (a unique compound of *C. odorata* with both allelopathic and defensive activities) and elicited stronger allelopathic effects on species native to China, the nonnative range of the invader, than on natives of Mexico, the native range of the invader.

• Our results suggest that invasive plants may evolve increased competitive ability after being introduced by increasing the production of novel allelochemicals, potentially in response to naïve competitors and new enemy regimes.

### Introduction

Many invasive plant species demonstrate greater competitive ability than natives (Ridenour *et al.*, 2008; Flory *et al.*, 2011; Vilà *et al.*, 2011; Qin *et al.*, 2013), which appears to contribute to the powerful impacts of invaders on the community composition, structure, and function of invaded ecosystems (D'Antonio & Kark, 2002; Rout & Callaway, 2009). A substantial component of the competitive ability and impact of invasive plants in their nonnative ranges appears to derive from much higher population densities and the greater growth of individuals than in their native ranges (Callaway *et al.*, 2012; Parker *et al.*, 2013). However, some invasive plants demonstrate stronger inhibitory effects even when high densities or greater biomass is accounted for (Callaway *et al.*, 2011; Inderjit *et al.*, 2011a; Qin *et al.*, 2013).

Some of the inherent competitive advantages of invasive plants are often studied in the context of the novel weapons hypothesis (NWH). The NWH predicts that unique chemicals of some invasive plants may provide disproportionately allelopathic, defense, or anti-microbial advantages in their nonnative ranges. With respect to allelopathy, novel chemicals may have stronger effects on naïve native plants from nonnative ranges of the invaders, which have not adapted to the unique chemicals, and therefore are more vulnerable to them than adapted plants from native ranges of the invaders (Callaway & Ridenour, 2004; Vivanco *et al.*, 2004; Thorpe *et al.*, 2009; Inderjit *et al.*, 2011a; Qin *et al.*, 2013; Svensson *et al.*, 2013).

Other studies attribute some portion of the greater competitive ability of invasive plants to the evolution of greater size after introduction (Blossey & Nötzold, 1995; te Beest *et al.*, 2009; Feng *et al.*, 2009). This evolutionary perspective is generally set in the context of the evolution of increased competitive ability (EICA), the hypothesis that some exotic species reallocate resources from defense to growth, and therefore increase competitiveness (Blossey & Nötzold, 1995). Some studies have found evidence for post-introduction evolution of increased competitive ability (Maron *et al.*, 2004; Ridenour *et al.*, 2008; Barney *et al.*, 2009; Flory *et al.*, 2011) or decreased defensive ability (Daehler & Strong, 1997; Wolfe *et al.*, 2004). But, as noted in a review by Bossdorf *et al.* (2005), relatively few studies have found evidence for both predictions of the EICA hypothesis simultaneously (Huang *et al.*, 2012; Qin *et al.*, 2013).

Responses to consumers can be complex, with some invaders reported to evolve increased defense against generalists in their nonnative ranges, while decreasing allocation to defense against specialists (Joshi & Vrieling, 2005; Wang et al., 2012; Liao et al., 2014), which is consistent with recent modifications of the EICA hypothesis (Müller-Schärer et al., 2004; Joshi & Vrieling, 2005). Müller-Schärer et al. (2004) proposed that, as invasive plants will still encounter generalists in their nonnative ranges but specialist herbivores will be absent, qualitative defense through toxins would be expected to increase (see Joshi & Vrieling, 2005; Ridenour et al., 2008; Liao et al., 2014), rather than decrease as predicted by the original EICA hypothesis. This is because high concentrations of toxins (to defend against generalists) no longer have the fitness costs of attracting specialist herbivores. Also, they noted that some plant toxins also function as allelopathic agents, potentially further enhancing the potential of a species to become invasive. Callaway & Ridenour (2004) proposed that, if novel plant toxins conferred competitive advantages in the nonnative ranges of invaders, then selection pressure for the toxins may be greater on the genotypes in the invaded regions than on the conspecific genotypes remaining at home. If so, the evolution of competitive ability might derive in some cases from selection for increased production of allelopathic compounds.

The inherent and evolved advantages of invasive plants are not mutually exclusive; invasive plants can evolve larger size, greater production of novel allelochemicals, or changes in qualitative and quantitative defense (Vivanco *et al.*, 2004; Ridenour *et al.*, 2008; Qin *et al.*, 2013). However, little effort has been made to explore how these processes might be integrated in invasion success (Bossdorf, 2013; Uesugi & Kessler, 2013).

Here we investigated the importance of integrating the EICA hypothesis and the NWH in the context of invasion by Chromolaena odorata, a native of the New World but a noxious invasive in much of the rest of the tropics and subtropics. In its native ranges, C. odorata harbors > 200 herbivores, and 25% of them are specialists (Zhang & Feng, 2007), whereas in its nonnative range in China few generalists and no specialists have been found on C. odorata (Xu et al., 2011). Odoratin (Eupatorium) is likely to be peculiar to C. odorata (Jiang & Chen, 1998; for details see the Materials and Methods section 'Materials'), which has strong allelopathic and defensive activities (Zhang, 2013). First, we compared the differences in intraspecific competitive ability and defense against aboveground enemies between C. odorata plants from nonnative and native populations in a common garden. Secondly, we conducted an enemy suppression experiment in order to determine the relative importance of above- and belowground enemies for the invader. Thirdly, we compared the allelopathic effects of rhizosphere soils of C. odorata plants from nonnative versus native ranges. We collected species from the nonnative and native ranges of the invader and compared their sensitivities to allelochemicals of the invader. Finally, we determined the difference in odoratin (Eupatorium)

content between *C. odorata* plants from native and nonnative ranges in a common garden.

### **Materials and Methods**

### Materials

Chromolaena odorata (L.) R. M. King and H. Robinson (Asteraceae) is a noxious invasive perennial herb/subshrub in much of the tropics and subtropics throughout the world. It is native to North, Central and South America, and was introduced to other tropical regions in the middle of the 19th Century. Chromolaena odorata is quite sparsely distributed in its native ranges, but can form dense mono-dominant stands in nonnative ranges, severely impacting agriculture, forestry, stockbreeding, biodiversity and environments of invaded areas (Goodall & Erasmus, 1996; Zhang & Feng, 2007). For example, the density of C. odorata was > 85 times higher in the nonnative range of China than in the native range of Mexico according to our field investigation at three sites in each country, but the height of individual plants did not differ between ranges (Supporting Information Fig. S1; Methods S1). In the nonnative range, individual C. odorata canopies decreased native plant richness by 41.2% across all sites investigated, whereas in the native range C. odorata did not affect or even increased native species richness (Fig. S2; Methods S1).

Odoratin (*Eupatorium*) ( $C_{19}H_{20}O_6$ ) is a type of chalcone. It was first isolated from C. odorata (then called Eupatorium odoratum) in 1973, and was not found in any other plants according to Jiang & Chen (1998). In fact, only two chalcones had been identified in plants from the big genus Eupatorium (with 1200 species) according to Yuan (2006). Phylogenetically related species are more likely to share secondary chemicals than unrelated species (Cappuccino & Arnason, 2006). Thus, odoratin (Eupatorium) is very likely to be peculiar to C. odorata. To further determine the uniqueness of this chemical to C. odorata, we searched SciFinder (Chemical Abstracts Service database) again in 2013, and still did not find any other plant that produces odoratin (Eupatorium). Four different chemicals were named 'odoratin' when they were first isolated from four different species with odorata or odoratum as the specific epithet. To distinguish the chemicals, Chemical Abstract added the genus name of the plant from which the chemical was first isolated in parentheses after 'odoratin'.

We collected seeds of *C. odorata* in 2009 from 15 populations in its native ranges (America; one from Jamaica, one from Cuba, two from Trinidad and Tobago, two from Puerto Rico, four from the USA, and five from Mexico) and 16 populations in its nonnative ranges (Asia; one from Malaysia, one from the Philippines, one from Thailand, two from Vietnam, two from Laos, and eight from China; Table S1). In each population, seeds were collected from 10 individuals which were at least 20 m apart, and saved separately by mother plant (10 seed families per population).

### Intraspecific competition experiment

To determine the differences in intraspecific competitive ability and defense against aboveground enemies between *C. odorata* 

plants from nonnative and native populations, we carried out a common garden experiment in Tlayacapan, Morelos, Mexico (18°57'N, 98°58'W; 1634 m above sea level (asl)), the native range of the invader. Here, the mean annual temperature is 19.3°C; the mean temperature of the hottest month (June) is 22.9°C and the mean temperature of the coolest month (January) is 16.9°C. The mean annual precipitation is 988.8 mm, with a dry period lasting from November to April (García, 1988). Seeds of 22 populations (11 nonnatives and 11 natives; Table S1) were selected randomly from the available seed pool, and sown separately into a seed bed in a glasshouse in October 2009. Maternal effects were not considered in our study, which did not influence biogeographic comparison results according to recent studies (Y-L. Zheng & Z-Y. Liao, unpublished) and Ridenour et al. (2008). In late December, when the seedlings were c. 5 cm tall, similar-sized vigorous seedlings were transplanted into two  $6 \times 18$  m blocks (Fig. S3), which were arranged parallel (1.2 m between the long sides of the blocks), and as much as possible we kept soil nutrients, moisture, and topography similar within and between the blocks. In each block we grew 220 C. odorata seedlings in monoculture (10 seed families × 22 populations) and 121 competing pairs (11 nonnative populations  $\times$  11 native populations; 6 cm between competitors). Each block was divided into 10 plots ( $6 \times 1.2$  m; 0.6 m apart along the long sides), and in each plot we grew 22 seedlings in monoculture (one from each of the 22 populations) and 11 competing pairs (one seedling from each of the 11 nonnative populations versus one seedling from each of the 11 native populations). Individual seedlings or competing pairs were 60 cm from any other seedling or seedling pair and were arranged randomly in each plot (three rows and 11 seedlings or seedling pairs per row). The two seedlings of each population in each plot (one for monoculture and one for competition) were from the same seed family, and the seedlings of each population in different plots (10) were from different seed families (10). The remaining 11 possible competing pairs were also grown in a row in each block. The arrangement of seedlings or seedling pairs in each plot and the arrangement of plots in each block were the same for the two blocks. To avoid border effects, we grew a protective row of C. odorata seedlings around each block (one row between the blocks). The seedlings were watered every other day at the rate of 2000 ml per seedling or seedling pair in the dry season. Weeds were removed when necessary.

Aboveground insect enemies were suppressed in one block by spraying seedlings or seedling pairs with a broad-spectrum insecticide with the main ingredients chlorpyrifos and permetrin (Foley Rey; National Agriculture Company, Magdalena Contreras, Mexico;  $2 \text{ ml l}^{-1}$  water). Belowground arthropods and fungi were suppressed by watering roots with a mixed solution of Foley Rey at  $2 \text{ ml l}^{-1}$ , Benomilo at  $2 \text{ g l}^{-1}$ , and Oxitrol DF at  $4 \text{ g l}^{-1}$ . Benomilo and Oxitro DF are two broad-spectrum fungicides (National Agriculture Company) with the main ingredients of benzimidazole and copper oxychloride, respectively. From February 2010 to the end of the experiment (November 2010), seedlings or seedling pairs were sprayed with Foley Rey solution every month (20 ml per seedling or seedling pair). From February to June, when the seedlings were small, each plant or plant pair was watered with 80 ml of the mixed solution every month. After June, 150 ml of the mixed solution was used for each plant or plant pair. Each plant or plant pair in the control block was sprayed and watered with an equal amount of water. These pesticides had no direct effects on the growth of *C. odorata* when used as described above (Fig. S4; Methods S2).

In October 2010, five *C. odorata* individuals (without competitors) per population grown in the block without enemy suppression and one branch per individual (with  $\approx$ 50 leaves) were randomly selected to measure leaf herbivory. The number of damaged leaves and the number of total leaves were counted for each individual, and the percentage of damaged leaves was calculated as the number of damaged leaves/the number of total leaves × 100%. Total leaf area and the area damaged by enemies were visually estimated by comparison with a paper square of dimensions 10 cm by 10 cm (accurate to 0.1 cm) for each leaf, and the percentage of damaged leaf area was calculated for each sample individual.

In November 2010, plant height was measured and then the aboveground parts of all plants were harvested, oven-dried at 60°C for 48 h, and weighed. Response to competition was measured by percentage change in biomass, that is, ((biomass<sub>comp</sub> – biomass<sub>mono</sub>)/biomass<sub>mono</sub>) × 100%, where biomass<sub>com</sub> and biomass<sub>mono</sub> are the mean aboveground biomass for each population when grown with and without competitors, respectively (Ridenour *et al.*, 2008; Qin *et al.*, 2013).

Effects of range, competition, enemy suppression, and their interactions on aboveground biomass were examined using threeway nested ANOVA. Population nested within range was used as a random factor. The differences in aboveground biomass of plants grown without competition in each enemy treatment, damaged leaves per plant, and damaged leaf area per plant between *C. odorata* plants from nonnative and native populations were examined using one-way nested ANOVAs. Range was used as a fixed factor; population nested within range was used as a fixed factor. The percentage changes in biomass caused by competition between *C. odorata* plants from nonnative and native ranges were compared using *t*-tests.

### Enemy suppression experiment

To determine whether aboveground or belowground consumers influenced *C. odorata* more in its native range, we conducted an enemy suppression experiment in the common garden in Tlayacapan, Morelos, Mexico. Seeds from 20 populations (10 nonnatives and 10 natives) were selected randomly from the available seed pool (Table S1) and sown separately into a seed bed in a glasshouse in October 2009. In December 2009, similar-sized seedlings (5 cm) were transplanted into four blocks ( $2.4 \times 24$  m; 1.2 m apart along the long sides) in the garden. Each block was divided into 10 plots ( $2.4 \times 1.8$  m; 0.6 m between neighboring plots), and in each plot we grew 20 seedlings, one per seed family per population. Individual seedlings were spaced 60 cm from one another and arranged randomly in each plot (four rows and five seedlings per row). The arrangement of seedlings in each plot and the arrangement of plots in each block were the same for the four blocks. To avoid border effects, we also grew a protective row of *C. odorata* seedlings around the blocks (one row between neighboring blocks). Each block was assigned randomly to one enemy treatment: aboveground enemy suppression; belowground enemy suppression; total enemy suppression; and no enemy suppression as the control. For enemy suppression treatments, we used the same methods as described in the intraspecific competition experiment. In December 2010, the aboveground parts of all plants were harvested, oven-dried at 60°C for 48 h, and weighed.

Effects of range, enemy treatment, and their interaction on aboveground biomass were examined using two-way nested ANOVA. Population nested within range and plot nested within block (enemy treatment) were used as random factors. The difference in biomass between *C. odorata* plants from nonnative and native ranges in the same enemy treatment was assessed using one-way nested ANOVA. Range was used as a fixed factor and population nested within range was used as a random factor. The differences in biomass among enemy treatments for *C. odorata* plants from each range were also assessed using one-way nested ANOVA. Enemy treatment was used as a fixed factor; plot nested within block was used as a random factor.

### Allelopathy experiment

To explore potential allelopathic effects of rhizosphere soils beneath C. odorata plants, four species native to China (Bidens biternata (Lour.) Merr. et Sherff, an annual herb; Galinsoga parviflora Cav., an annual herb; Inula cappa (Buch.-Ham.) DC., a perennial shrub; and Laggera pterodonta (DC.) Benth., a perennial herb) and four species native to Mexico (Cosmos sulphureus Cav., an annual herb; Chrysanthemum leucanthemum L., a perennial herb; Lasianthaea crocea (A. Gray) K. M. Becker, a perennial subshrub; and Tithonia tubiformis (Jacq.) Cass., a perennial subshrub) were used as test species. We chose Asteraceae species with three different life forms in each range, that is: annual herb, perennial herb and perennial shrub. These species are common in Yunnan and Morelos, and co-occur with C. odorata. Seeds of the four species native to China were collected in Yunnan Province in April 2012; seeds of the four species native to Mexico were collected in Morelos in March 2012. Seeds were collected from > 10 individuals of each species, the individuals being at least 20 m from each other.

In June 2010, *C. odorata* seeds from 10 nonnative and 10 native populations were selected randomly from the available seed pool (Table S1) and sown separately into a seedbed in a glasshouse in China. In August 2010, when the seedlings were 10 cm tall, seedlings were planted in the common garden in Menglun, Yunnan Province, southwest China (21°56'N, 101°150'E; 570 m asl). Here the mean annual temperature is 21.7°C, the mean temperature in July (the hottest month) is 25.3°C, and the mean temperature in January (the coolest month) is 15.6°C. The average annual precipitation is 1557 mm with a dry period lasting from November to April. In August 2012, rhizosphere soils were collected from three native and three nonnative populations (Table S1). Soils from each population was mixed uniformly, and put into 16 pots, 200 g per pot. To

reduce potential allelopathic effects of the soils, pure activated carbon (AC; SCM Industrial Chemical Co. Ltd, Shanghai, China) was added to half of the pots (5 ml per pot) and mixed uniformly (Lau *et al.*, 2008). Seeds of each test species were added to two pots (100 seeds per pot), one with and one without AC. One month later, we calculated the seed germination rate and measured seedling length (shoot and root). The potential allelopathic effects of the rhizosphere soils from each population on each test species were calculated as (performance in soil without AC – performance in soil with AC)/performance in soil with AC.

Activated carbon may also influence growth of some plants via its effects on soil nutrient availability, such as direct addition of nutrients with AC (Lau *et al.*, 2008). Thus, we used pure AC, and measured the seed germination rate in addition to seedling length.

Effects of *C. odorata* range, test species origin, and their interaction on percentage changes in germination rate and seedling growth were examined using two-way nested ANOVA. Population of *C. odorata* nested within range and test species nested within origin were used as random factors. Differences in the allelopathic effects of *C. odorata* plants from nonnative versus native ranges on test species from each range were assessed using one-way nested ANOVA. Range was a fixed factor and population nested within range was a random factor. The difference in the allelopathic effects of *C. odorata* plants from each range on test species from Mexico versus China was also examined using one-way nested ANOVA. The origin of test species was a fixed factor and test species nested within origin was a random factor.

### Biogeographic difference in odoratin (Eupatorium)

In April 2013, newly mature leaves of C. odorata were collected from five plants of each of the 10 native and 10 nonnative populations (Table S1) grown in the common garden established in Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences. The leaves from each plant were dried separately under room temperature (five replicates per population), and ground. The powder (500 mg) was extracted using 50 ml of methanol for 24 h. Odoratin content in the extract was determined using ACQUITY Ultra Performance Liquid Chromatography (Waters Corp., Miller, MA, USA), equipped with a BECH C18 column  $(2.1 \text{ mm} \times 50 \text{ mm}, 1.7 \mu\text{m}; \text{Waters Corporation})$ . The mobile phase included (A) pure water and (B) acetonitrile. The concentration of eluent B was changed from 10% to 60% within 10 min (linear gradient). The flow rate of the eluent was  $0.6 \text{ ml min}^{-1}$ , the injection volume of the extract was 5  $\mu$ l, and the column oven was set at 25°C. The conditions for mass spectrometric detection were as follows: electrospray ionization (ESI) was performed in positive ion mode at 1.8 kV, the ion source temperature was 350°C, the solvent temperature was 550°C, the sheath gas flow rate was  $8001h^{-1}$ , and the auxiliary gas flow rate was  $1501h^{-1}$ . All MS/MS data for odoratin were collected using multiple reaction monitoring (MRM) of the transition m/z 367.10, m/z 352.08, m/z 233.07 under the conditions: cone voltage 58.0, collision energy 16.0, dwell time 0.003 s, and ionization mode

ESI<sup>+</sup>. All measurements were obtained in the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

The difference in odoratin (*Eupatorium*) content between *C. odorata* plants from the nonnative and native ranges was assessed using one-way nested ANOVA in which range was a fixed factor and population nested within range was a random factor.

### Results

#### Biogeographic differences in competition and defense

Without competition in the common garden in the native range of Mexico, C. odorata plants from the native range produced 29.3% more aboveground biomass than plants from the nonnative range in the block with enemy suppression (for range,  $F_{1,20} = 5.395;$  P = 0.031; for population,  $F_{20,192} = 3.268;$ P < 0.001), but not in the block without enemy suppression (for range,  $F_{1,20} = 0.638$ ; P = 0.434; for population,  $F_{20,193} = 2.308$ ; P = 0.002; Fig. 1). The results were consistent with the significant interaction between range and enemy treatment (Table S2), and indicated that C. odorata plants from the native range benefited more from suppressing natural enemies than C. odorata plants from the nonnative range. Indeed, either with or without competition, suppressing natural enemies significantly increased aboveground biomass for C. odorata plants from the native range (without competition,  $F_{1,189} = 12.745$ ; P < 0.001; with competition,  $F_{1,442} = 11.027$ ; P < 0.001) but not for plants from the nonnative range (without competition,  $F_{1,196} = 0.368$ ; P = 0.545; with competition,  $F_{1,446} = 1.201$ ; P = 0.274; Fig. S5).

Intraspecific competition significantly decreased the biomass of *C. odorata* plants from both ranges (Fig. 1, Table S2), but decreases were 49.3% and 30.7% greater for *C. odorata* plants from the native range than for plants from the nonnative range in blocks with (for range,  $t_{20} = 2.253$ ; P = 0.036) and without (for range,  $t_{20} = 2.153$ ; P = 0.044) enemy suppression, respectively. The results were consistent with the significant interaction between range and competition (Table S2).

In the common garden in the native range of Mexico, herbivores preferred *C. odorata* plants from the nonnative range to *C. odorata* plants from the native range. The percentage of damaged leaves (for range,  $F_{1,20}=7.834$ ; P=0.011; for population,  $F_{20,88}=9.266$ ; P<0.001) and percentage of damaged leaf area (for range,  $F_{1,20}=22.284$ ; P<0.001; for population,  $F_{20,88}=3.376$ ; P<0.001) were 58.5% and 120.3% higher for plants from the nonnative range than for plants from the native range, respectively (Fig. 2). *Sphenarium purpurascens*, a generalist and the main aboveground consumer observed in the garden in Mexico, also preferred eating *C. odorata* plants from the nonnative range in a feeding choice experiment (Fig. S6; Methods S3).

### Differences in effects of above- versus belowground enemies on growth

Effects of range, enemy treatment, and their interaction on aboveground biomass were significant (Table S3). *Chromolaena* 





Range

**Fig. 1** Biomass of *Chromolaena odorata* plants from the native (open bars) and nonnative (closed bars) populations grown in monoculture, and change in biomass caused by intraspecific competition in the blocks without enemy suppression (a) and with enemy suppression (b). Narrow bars indicate the mean + 1 SE (n = 10 for monoculture; n = 11 for competition) for each population; two thicker bars in the center depict the mean + 1 SE for each range. Significant differences between ranges (\*, P < 0.05) in biomass (one-way nested ANOVAs) and percentage change in biomass (*t*-test) are shown.

odorata plants from the native range produced similar aboveground biomass to plants from the nonnative range in the block without enemy suppression (for range,  $F_{1,18} = 4.454$ ; P = 0.053; for population,  $F_{18,176} = 1.421$ ; P < 0.127; Fig. 3). However, C. odorata plants from the native range produced 17.15%, 31.62%, and 32.21% more biomass than C. odorata plants from the nonnative range in blocks with aboveground enemy suppression (for range,  $F_{1,18} = 5.751$ ; P = 0.027; for population,  $F_{18,171} = 2.128$ ; P < 0.007), belowground enemy suppression (for range,  $F_{1,18} = 15.976; P = 0.001;$  for population,  $F_{18,171} = 2.506;$ P=0.001), and total enemy suppression (for range,  $F_{1,18} = 16.478$ ; P = 0.001; for population,  $F_{18,173} = 2.551$ ; P < 0.001), respectively. The effect of aboveground enemy suppression was not significant for C. odorata plants from both ranges. Suppression of belowground or total enemies significantly increased biomass for C. odorata plants from the native range but not for plants from the nonnative range (Fig. 3). For C. odorata

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**Fig. 2** Damaged leaves per plant (a) and damaged leaf area per plant (b) for *Chromolaena odorata* plants from native (open bars) and nonnative (closed bars) populations. Narrow bars indicate the mean + 1 SE (n = 5) for each population; two thicker bars in the center depict the mean + 1 SE for each range. Significant differences between ranges (one-way nested ANOVAs): \*, P < 0.05; \*\*\*, P < 0.001.

plants from the native range, fungicide and insecticide applied to the soil increased aboveground biomass by 24.8% (P < 0.05), whereas the suppression of aboveground enemies had no significant effect on growth (Fig. 3). Suppression of belowground enemies and suppression of both below- and aboveground enemies did not differ significantly in their effects on growth.

### Biogeographic differences in allelopathy

Both the range of *C. odorata* and the origin of the test species influenced seed germination and seedling growth significantly (Table S4). Rhizosphere soils from *C. odorata* plants grown from seeds collected in the nonnative range more strongly inhibited seed germination and seedling growth of the test species native to either Mexico or China than rhizosphere soils from *C. odorata* plants originating from the native range (Fig. 4; Table S5). Also, the germination and seedling length of the four species native to China (the nonnative range of *C. odorata*) were more inhibited by rhizosphere soils of *C. odorata* plants originating from either the nonnative or the native range than those of the four species native to Mexico, the native range of *C. odorata* (Fig. 4; Table S6).

### Biogeographic difference in odoratin (Eupatorium) content

Odoratin (*Eupatorium*) content was on average 140.7% higher in leaves of *C. odorata* plants from the nonnative range than in

 $\begin{array}{c}
400\\
\hline
5\\
300\\
\hline
8\\
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8\\
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9\\
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100\\
0\\
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None Above Below Total Enemy suppression
\end{array}$ 

**Fig. 3** Biomass of *Chromolaena odorata* plants from the native (open bars) and nonnative (closed bars) populations grown in the blocks with aboveground enemy suppression (Above), belowground enemy suppression (Below), total enemy suppression (Total), and no enemy suppression (None). Different small or capital letters indicate significant differences among enemy treatments for plants from the same range (P < 0.05; one-way ANOVA); significant differences between plants from the native and nonnative ranges in the same enemy treatment (one-way nested ANOVAs): \*, P < 0.05; \*\*\*, P < 0.001.

plants from the native range (for range,  $F_{1,18} = 10.655$ ; P = 0.004; for population,  $F_{18,77} = 52.327$ ; P < 0.001; Fig. 5).

### Discussion

In common conditions, C. odorata plants grown from seeds collected from the nonnative range were less affected by competition than plants from the native range, either with or without the experimental manipulation of consumers. But C. odorata plants from the nonnative range were not larger in the field (Fig. S1; Methods S1) and were smaller in two common garden experiments. Instead, plants from the nonnative ranges produced much more odoratin (Eupatorium), a potential novel allelochemical, in the nonnative range. With regard to the second component of EICA, C. odorata plants from the nonnative range exhibited weaker defense against aboveground enemies but increased defense against belowground enemies. Thus, our results are consistent with the general predictions of the EICA hypothesis, but with a twist. We found evidence for decreased allocation to defense, but not increased allocation to growth and thus any competitive advantages that might correspond to growth. Instead, we found that C. odorata plants from the nonnative ranges on average allocated more to the production of odoratin (Eupatorium), and perhaps to increased allelopathic effects (see Callaway & Ridenour, 2004; Ridenour et al., 2008).

The inherent advantages of simply possessing a novel allelochemical, as well as the evolution of stronger allelopathy, may help to explain the stronger competitive ability detected in our intraspecific (Fig. 1) and interspecific (Qin *et al.*, 2013) competition experiments. Allelopathy of rhizosphere soils of *C. odorata* plants inhibited seed germination and seedling growth of species native to China (the nonnative range of the invader) more than those of species native to Mexico (the native range of the invader) (Fig. 4), suggesting that native species from China were more vulnerable to allelochemicals released to rhizosphere soil by the invader than natives from Mexico. The result was consistent with the novelty of odoratin (*Eupatorium*) in the invasive range of *C. odorata*, and provided a possible explanation for the pattern that



Fig. 4 Allelopathic effects of rhizosphere soils of Chromolaena odorata plants from native (open bars; white or gray) and nonnative (horizontal bars; white or gray) populations on seed germination (a) and seedling growth (b) of test species native to Mexico (white bars; open or horizontal) and China (gray bars; open or horizontal). Narrow bars indicate the mean + 1 SE (n = 3) for each native species; four thicker bars in the center depict the mean + 1 SE for all native species from each range. Significant differences in allelopathic effects on species native to Mexico or China between C. odorata plants from native and nonnative populations: \*, P < 0.05 (one-way nested ANOVA); different small or capital letters indicate significant differences in allelopathic effects of C. odorata plants from the native or nonnative range on species native to China versus species native to Mexico. (\*, P < 0.05; one-way nested ANOVA) C. s, Cosmos sulphureus; C. I, Chrysanthemum leucanthemum; L. c, Lasianthaea crocea; T. t, Tithonia tubiformis; B. b, Bidens biternata; G. p, Galinsoga parviflora; I. c, Inula cappa; L. p, Laggera pterodonta.

C. odorata had much stronger impacts on native plants in the nonnative range in China than in the native range in Mexico, even when calibrating for density by sampling under individual canopies (Fig. S2; Methods S1). Thus, allelopathy seems to provide an inherent competitive advantage to C. odorata in its nonnative range, regardless of any evidence of increased concentrations. But we also found that plants from the nonnative range produced on average 2.4 times as much odoratin (Eupatorium) in their leaves as plants from the native range. This difference may have arisen from natural selection favoring genotypes with stronger chemical effects – if allelochemicals provide greater competitive advantages in the nonnative range than in the native range (see Ridenour et al., 2008) - or through the introduction of genotypes from specific populations in the native range with high odoratin (Eupatorium) content. For example, plants originating from seeds collected from three of the four sites (populations; Fig. 5; Table S1) in Florida produced as high or higher odoratin (Eupatorium) compared with the mean of populations from the



**Fig. 5** Odoratin (*Eupatorium*) contents of *Chromolaena odorata* plants from native (open bars) and nonnative (closed bars) populations grown in a common garden in China. Narrow bars indicate the mean + 1 SE (n = 5) for each population; two thicker bars in the center depict the mean + 1 SE for each range. Significant differences between ranges: \*\*\*, P < 0.001 (one-way nested ANOVA).

nonnative range. However, plants originating from Trinidad had much lower odoratin (*Eupatorium*) concentration than plants from nonnative populations. Both the populations from Florida and Trinidad could be the source for *C. odorata* invasions in Asia, but this is not yet known (see Qin *et al.*, 2013).

Leaf leachate directly applied to seeds of species native to Mexico and China produced far stronger effects than our test of allelopathy via rhizosphere soils in the present study (Fig. 4 versus Qin *et al.*, 2013). Indeed, allelopathic effects are often not detected at all via isolated soils even when leachates have been shown to have powerful inhibitory effects (Pollock *et al.*, 2009; Inderjit *et al.*, 2011b). Thus, our tests of potential allelopathic effects are probably conservative.

The EICA hypothesis causally links evolutionarily decreased allocation of resources to defense to the increased growth and therefore increased competitive ability of introduced plants (Blossey & Nötzold, 1995; Feng et al., 2009). However, the stronger competitive ability of C. odorata plants from the nonnative range was not accompanied by larger individual size, suggesting a minor role of decreased resource allocation to defense in increased competitive ability. Certainly, we cannot exclude other trait changes that could lead to greater competitive ability, but C. odorata plants from the nonnative range were smaller than plants from the native range in common gardens (Figs 1b, 3; Qin et al., 2013). In addition, C. odorata plants from the nonnative range did not decrease defense against soil-borne enemies. Chromolaena odorata has also been reported to have evolved increased defense against Ganesella saurivonga, a generalist snail, which occasionally damages C. odorata in China, and other two aboveground generalists (Helicoverpa armigera and Prodenia litura; Liao et al., 2014). The results were consistent with the higher odoratin (Eupatorium) contents of C. odorata plants from nonnative populations, as H. armigera and P. litura were vulnerable to odoratin (Eupatorium) (Zhang, 2013). Sphenarium purpurascens and other aboveground herbivores in Mexico may not be sensitive to odoratin (Eupatorium), as

*C. odorata* plants from the nonnative populations showed lower defense against them (Figs 2, S6; Methods S3). Our results suggest that evolutionary responses to generalists and sensitivity to chemicals may be species-specific.

Our results support the theory first proposed by Müller-Schärer *et al.* (2004) as a modification of EICA to better differentiate between specialist and generalist consumers. *Chromolaena odorata* plants from the nonnative range increased defense against soil-borne enemies (Fig. 3) and three aboveground generalists (Liao *et al.*, 2014), consistent with their higher contents of odoratin (*Eupatorium*) (Fig. 5) and phenolics (Liao *et al.*, 2013). Odoratin (*Eupatorium*) also has a strong defensive ability against generalists (Zhang, 2013). However, *C. odorata* plants from nonnative ranges may have decreased defense against specialists as they have lower cellulosic contents and toughness than plants from native populations (Liao *et al.*, 2013).

To our knowledge, almost all studies testing the EICA hypothesis have focused on aboveground herbivores (Funk & Throop, 2010; Huang et al., 2012; Williams et al., 2010; Wang et al., 2012; Qin et al., 2013; but see Beckstead & Parker, 2003). Our results provide a rare biogeographic comparison of soil-borne enemies in the context of EICA. Chromolaena odorata plants from the native range were more vulnerable to belowground enemies. However, C. odorata plants do not suffer from fewer soilborne enemies in the nonnative range than in the native range (te Beest et al., 2009). Interestingly, C. odorata plants accumulate native soil-borne pathogens in the nonnative range in India under some conditions (Mangla et al., 2008), and, although C. odorata did not appear to respond as negatively to these pathogens as native species, this provides a scenario in which natural selection may favor increased defense against soil-borne enemies such as our evidence suggests.

Suppressing aboveground enemies did not promote the growth of *C. odorata* plants from either range, and nor did aboveground natural enemies decrease the superior competitive ability of *C. odorata* plants from the nonnative range, even though they were damaged more severely by aboveground enemies. The low level of aboveground herbivory (3–7%) may not have been heavy enough to strongly influence plants from either range, as *C. odorata* appears to be highly tolerant to aboveground herbivory (Li *et al.*, 2012).

In conclusion, naïve native species from China were more vulnerable to rhizosphere soils and to novel odoratin (*Eupatorium*) than coadapted natives from Mexico, which may have promoted directional selection on genotypes with high allelopathic effects. New enemy regimes (presence of generalists and absence of specialists) in introduced ranges may also exert directional selection on genotypes with high odoratin (*Eupatorium*) content, which had strong abilities to defend against generalists. It has been recognized that other defensive chemicals also have allelopathic activities (Callaway *et al.*, 2008; He *et al.*, 2009; Inderjit *et al.*, 2011a), and thus selection pressure for strong defense may also favor genotypes with high allelopathy. Our results suggest that *C. odorata* may evolve increased competitive ability after being introduced by increasing the production of novel odoratin (*Eupatorium*), rather than by increasing

individual size. Our results integrate the EICA hypothesis and NWH, providing a potential novel explanation for the increased competitive ability of introduced plants and emphasizing the importance of integrating different mechanisms to understand exotic plant invasions.

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### **Supporting Information**

Additional supporting information may be found in the online version of this article.

Fig. S1 Density and height of individuals for *Chromolaena odorata* plants in the native and nonnative ranges.

Fig. S2 Native plant richness in the quadrats with and without *Chromolaena odorata* in the native and nonnative ranges.

Fig. S3 Schematic diagram showing planting design in the intraspecific competition experiment.

Fig. S4 Effects of pesticides Foley Rey, Benomilo and Oxitrol on growth of *Chromolaena odorata*.

Fig. S5 Effects of suppressing above- and belowground enemies on biomass for *Chromolaena odorata* plants from the same range.

**Fig. S6** Absolute leaf area and leaf area fraction consumed by a grasshopper in choice feeding experiments for *Chromolaena odorata* plants from native and nonnative populations.

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 Table S1 Background information on sampled populations of

 Chromolaena odorata

**Table S2** Effects of range, competition, enemy suppression, and their interactions on biomass of *Chromolaena odorata* plants in the intraspecific competition experiment

**Table S3** Effects of range, enemy suppression, and their interaction on biomass of *Chromolaena odorata* plants in the enemy suppression experiment

**Table S4** Effects of *Chromolaena odorata* range, test species origin, and their interaction on changes in germination rate and seedling growth in the allelopathy experiment

**Table S5** Effects of range of *Chromolaena odorata* on changes in germination rate and seedling growth for test species from Mexico or China in the allelopathy experiment

**Table S6** Differences in sensitivity to allelochemicals of*Chromolaena odorata* plants from the native or nonnative rangebetween test species from Mexico and China

**Methods S1** Biogeographical differences in *Chromolaena odorata* performance and its impact on plant richness in the field

Methods S2 Effects of three pesticides on *Chromolaena odorata* growth

Methods S3 Choice feeding of a grasshopper on *Chromolaena odorata* plants from native versus nonnative populations

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