

Volatile chemicals from leaf litter are associated with invasiveness of a Neotropical weed in Asia

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Abstract. Some invasive plant species appear to strongly suppress neighbors in their nonnative ranges but much less so in their native range. We found that in the field in its native range in Mexico, the presence of *Ageratina adenophora*, an aggressive Neotropical invader, was correlated with higher plant species richness than found in surrounding plant communities where this species was absent, suggesting facilitation. However, in two nonnative ranges, China and India, *A. adenophora* canopies were correlated with much lower species richness than the surrounding communities, suggesting inhibition. Volatile organic compound (VOC) signals may contribute to this striking biogeographical difference and the invasive success of *A. adenophora*. In controlled experiments volatiles from *A. adenophora* litter caused higher mortality of species native to India and China, but not of species native to Mexico. The effects of *A. adenophora* VOCs on seedling germination and growth did not differ between species from the native range and species from the nonnative ranges of the invader. Litter from *A. adenophora* plants from nonnative populations also produced VOCs that differed quantitatively in the concentrations of some chemicals than litter from native populations, but there were no chemicals unique to one region. Biogeographic differences in the concentrations of some volatile compounds between ranges suggest that *A. adenophora* may be experiencing selection on biochemical composition in its nonnative ranges.

Key words: *Ageratina adenophora*; *allelopathy*; *Bambusa arundinacea*; *Bidens biternata*; *biogeography*; *China*, *India*, and *Mexico field sites*; *litter*; *native species richness*; *novel-weapons hypothesis*; *plant invasion*; *volatile organic chemicals*.

INTRODUCTION

Why some plant species are able to attain near monocultural dominance in communities where they are exotic but not where they are native remains poorly understood (Callaway and Maron 2006). But this biogeographic shift in dominance is the essence of exotic “invasion” and in the absence of disturbance, strong competitive interactions are likely to play an important role in establishing the dominance of invaders (Levine et al. 2003, Vilà and Weiner 2004, Maron and Marler 2008, Munshaw and Lortie 2010). Release from specialist herbivores or pathogens may allow plants to be more competitive in nonnative ranges (Keane and Crawley

2002). Successful invaders may possess competitive advantages because they come from a more competitive species pool or happen to possess inherent traits that give them an advantage relative to their new neighbors. One explicit hypothesis for why some species become more competitively dominant in their new ranges is the “novel-weapons hypothesis” (NWH), which poses that some invaders possess allelopathic, anti-herbivore, or antimicrobial chemicals to which native organisms have not adapted, and that these novel compounds provide invaders with direct or indirect competitive advantages (Callaway and Aschehoug 2000, Callaway and Ridenour 2004). Evidence for the NWH comes from experiments in which chemicals produced by invaders were applied to species from invaded and native ranges (Callaway et al. 2008, He et al. 2009), studies comparing allelopathic and defense interactions among invaders and native plants

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(Wikström et al. 2006, Jogesh et al. 2008, Ens et al. 2009a, b, Jarchow and Cook 2009), and literature-based comparisons of the novelty of chemicals produced by invasive species relative to chemicals identified in the native flora (Cappuccino and Arnason 2006).

Early studies of allelopathy focused on volatile organic compounds (VOCs) (Muller et al. 1964, Muller 1965), but interest in VOCs as agents of allelopathy faded after their ecological importance in the field was challenged by experiments showing that herbivores caused the patterns attributed to VOCs (Bartholomew 1970). Recently, VOCs have gained attention in other ecological roles as factors in communication among plants (Karban 2007, Karban and Shiojiri 2009), signals to the predators of herbivore enemies (Kessler and Baldwin 2002), and in self–nonself recognition (Eom et al. 2006). And they have reemerged as potential factors in allelopathic interactions as well (Barney et al. 2005, Eom et al. 2006, Karban 2007).

A plant species that may employ VOCs in allelopathy is *Ageratina adenophora* (Sprengel) R. M. King and H. Robinson [syn. *Eupatorium adenophorum*, Asteraceae], a species producing a large number of volatiles with broad biocidal properties (Palá-Paúl et al. 2002). *Ageratina adenophora* is a shrub native to central Mexico but invasive in subtropical climates throughout the world including Africa, India, the Philippines, Europe, China, and Australia. *Ageratina adenophora* can establish virtual monocultures where diverse native communities once flourished (Wang and Wang 2006) and appears to be expanding its range in the areas of our study sites in India and China (Zhu et al. 2007). In addition to bioactivity of its VOCs, there is some evidence for *A. adenophora* allelopathy based on tissue extracts and field applications of activated carbon (Song et al. 2000, Zhang et al. 2008). These results plus the powerful and unusual smell of its litter make *A. adenophora* an interesting candidate for comparing the biological effects of VOCs on competitors from both native and invasive ranges to gain insight into whether allelopathy might play a role in its invasive abilities.

METHODS

Effect of Ageratina adenophora in the field

There were two field sites in the native range, one in northeast Mexico in the Sierra Madre Oriental near Balcón de Moctezuma (1400 m above sea level; 23°36' N, 99°13' W) in the state of Tamaulipas, and the other in central Mexico in the state of Querétaro (1740 m a.s.l.; 19°52' N, 100°54' W). In India, we sampled two sites in Mussoorie (30°28.750' N, 78°03.216' E; 1663 m elevation, and 30°28.272' N, 78°03.463' E; 1866 m, respectively). In China, one site was located at Xujiaba (2430 m a.s.l.; 24°32' N, 101°1' E) and a second at Taizhong (1420 m a.s.l.; 24°27' N, 100°54' E). Both sites were in Jingdong County, Yunnan Province, and were 15 km from each other. At each site in India we randomly placed 20, 1 × 1 m quadrats in large patches of *Ageratina*

adenophora and 20 quadrats in random locations without *A. adenophora*, but near to and surrounding the patches in areas with similar soil type, soil moisture, and topography. In India we counted the number of different species in each quadrat. At each site in Mexico and in China we randomly located 20 shrubs and then randomly located 1 × 1 m quadrat under each canopy. Each quadrat under *A. adenophora* canopies was paired with a quadrat in the open. In Mexico and China the cover of each species was estimated in each quadrat in percent cover classes of 1%, 2%, and then in 5% increments from 5% through 100% (e.g., 10%, 15%, 20% and so on).

VOC bioassay

We first tested the effect of VOCs emitted from *A. adenophora* leaf litter on the germination of *Bidens biternata* and *Bambusa arundinacea* seedlings at the University of Delhi (Delhi, India). *Bambusa arundinacea* is native to India and widely distributed (Mangla et al. 2008). *Bidens biternata* is distributed and naturalized throughout India (Dakshini and Singh 1984). Eight seeds of either *B. biternata* or *B. arundinacea* were placed on Whatman filter paper number 1 in each of three 7.5–8.0 cm diameter glass petri dishes. To maintain uniform moisture, a thin layer of cotton soaked in 10 mL distilled water was placed below the filter paper. Three petri dishes were then placed in glass chambers (interior diameter 190, height 100 mm) at the University of Delhi, with either 20 g of *A. adenophora* leaf litter, collected as it fell naturally in the field at our Indian field sites and then air-dried, or empty chambers as controls. Chambers were then covered with glass lids. Twenty grams of *A. adenophora* litter approximated realistic depths of litter observed in the field. For each species and treatment combination there were five glass chambers, thus five replicates. The chambers were incubated at 28–32°C and a 12 h:12 h light : dark cycle. Seed germination was counted daily and root and shoot lengths were measured after 7 days (*B. biternata*) or 8 days (*B. arundinacea*). Mean root or shoot lengths were compared with *t* tests.

Biogeographic comparisons

Ecologically realistic concentrations of VOCs are very difficult to determine, thus comparing allelopathic effects on species from native and nonnative ranges of invaders adds to the argument for the potential ecological relevance of the interaction as well as yielding evidence for whether or not allelopathy might contribute to invasive success. We collected seeds in the field in communities with *A. adenophora* in Mexico, China, and India. We planted these seeds in 200-cm³ “rocket pots” containing 5 mL of soil and 25 mL of silica sand. Pots were then placed in 115-L (84 × 40 × 35 cm) transparent plastic bins that contained either 1.5 L of *A. adenophora* litter collected from Indian populations, or a 1.5-L mixture of field-collected litter from species that do not occur in any of the three ranges, *Quercus macrocarpa*,

Acer platanoides, and *Populus tremuloides*, collected on The University of Montana campus (Missoula, Montana, USA). We used litter from these other species as a control because we did not know what general effects decaying litter might have on CO₂ concentrations or concentrations of other volatile substances; thus we were attempting to discriminate between the effects of chemicals emitted from *A. adenophora* litter and the potential effects of other gases that might be emitted from litter in general. None of these three species is known to exert allelopathic effects on other species, nor do we know of measurements of VOCs from these species.

We initially used seeds of 16 species from Mexico, nine species from China, and eight species from India, but germination was high enough to test mortality rates for only five, seven, and seven species, respectively. For each of the 33 species, we planted 30 rocket pots with five seeds each for a total of 990 pots. Three pots of each species were placed in each of 10 bins; five bins contained *A. adenophora* litter while the other five bins contained the Montana litter, as described above. Litter was air-dried for two days and then placed in the bottom of the bins; rocket pots were placed in racks that suspended the rocket pots above the litter without touching. Bins were covered with lids leaving 120 cm² of each cover open, allowing circulation but ensuring that air within the bins was affected by VOCs; this was attested to by the exceptionally strong smell within the bins and throughout the greenhouse. During the experiment germination was counted as plants emerged but new germinants were thinned so that the survival and final mass of the first germinant was measured in each rocket pot. These seedlings were grown in a greenhouse from 23 January 2008 to 21 March 2008 with ambient light supplemented by metal halide bulbs, keeping photosynthetically active radiation (PAR) during the day above 1200 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ with a day length of 13 hours. We watered plants every two to three days. After 59 days the final total dry biomass of all individuals, dried at 60°C for three days, was measured and mortality was recorded. We analyzed the biomass of individual plants and the mean germination percentage within pots with ANOVAs using region and site as fixed variables, bin as a block, and species as nested random variables within a site. We analyzed mortality by calculating the mean mortality for each species in each treatment across all bins, and then using a one-way ANOVA to compare the means of all species from both nonnative ranges to the means of all species from the native range.

Identification and quantification of volatiles

We grew *A. adenophora* from seeds of nine separate populations collected in each of three different countries, Mexico, China, and India. Plants were grown side by side in a greenhouse at the Max Planck Institute for Chemical Ecology (Jena, Germany) with additional

illumination by sodium lamps. Temperature was maintained at 20–22°C during a 16-h light period and at 18–20°C during the night; relative humidity was 30–55%. Plants were potted in commercial soil (Tonsubstrat; Klasmann, Geeste/Groß-Hesepe, Germany) and watered every day with tap water. Five months after germination leaves of plants from three populations of the three different origins were collected and dried for 10 d at room temperature (~25°C). Dried leaves were homogenized using metal beads with agitation by a paint shaker two times for three minutes each. To collect VOCs from homogenized *A. adenophora* leaves, we applied a closed-loop stripping method for headspace analysis. We placed 200 mg of homogenized leaves in a 1-L desiccator and VOCs were collected for 2 h by recirculating desiccator air through a charcoal trap with a pump. VOCs were eluted from the trap with 60 μL of ethyl acetate containing 20 ng/ μL nonyl acetate as an internal standard (IS).

Plant material from the same harvest was also used for direct solvent extraction of potential VOCs. Here 200 mg homogenized leaves were extracted with 1 mL of ethyl acetate containing 20 ng/ μL IS for 24 h at room temperature under continuous rotation. Solvent extracts were purified and dried over Na₂SO₄. All extractions were performed as four replicates on three populations from each of the three regions. Products of volatile collection and solvent extractions were identified by gas chromatography (Agilent Hewlett-Packard 6890; Agilent Technologies, Santa Clara, California, USA) coupled to a mass selective detector (MS) (Agilent Hewlett-Packard 5973) or a flame ionization detector (FID). For analyses, 2 μL of ethyl acetate extracts were injected at 230°C. Compounds were separated on a DB5-MS column (30 m \times 0.25 mm \times 0.25 μm ; J & W Scientific, Santa Clara, California, USA) with temperature programmed at 40°C for 2-min hold rising to 175°C at the rate of 3°C/min, and to 250°C at the rate of 90°C/min with a 3-min final hold. The flow rate of carrier gas for GC-MS (helium) and GC-FID (hydrogen) was 1 and 2 mL/min, respectively. All terpene products were identified by using Agilent Technologies software (Santa Clara, California, USA) with the Wiley 275.L and NIST 98.1 mass spectra libraries, as well as by comparison of mass spectra and retention times with those of authenticated standards where available.

RESULTS

Ageratina adenophora canopies and understory communities in the field

For the sites tested separately in its native range in Mexico, *Ageratina adenophora* canopies had no significant relationship with quadrat-based species richness (Fig. 1), but across both sites combined, with site as a factor in the analysis, *A. adenophora* canopies were significantly correlated with higher species richness than surrounding vegetation, suggesting a facilitative effect (site \times treatment ANOVA; $F_{\text{trt}} = 5.45$; $\text{df} = 1, 76$; $P =$

0.022). In contrast, species richness under *A. adenophora* canopies at all sites in the invaded ranges of China and India was much lower than in the open where there was no *A. adenophora*. In India, at Mussoorie site 1 species richness was 85% lower under *A. adenophora* (7.5 ± 0.3 to 1.2 ± 0.2 species/m² [means \pm SE]; *t* test, *df* = 1, 38; *t* = 20.76; *P* < 0.0001), and at Mussoorie site 2 species richness was 69% lower (20.7 ± 0.7 vs. 6.4 ± 0.7 species/m²; *df* = 1, 38; *t* = 4.96; *P* < 0.0001; Fig. 1) under *A. adenophora* than where the invader was not present. In China, at Xujiaba *A. adenophora* was associated with 53% lower species richness than the surrounding vegetation (11.8 ± 0.6 vs. 5.6 ± 0.6 species/m²; *df* = 1, 38; *t* = 13.11; *P* < 0.0001), and at Taizhong the richness was 64% lower under *A. adenophora* (10.7 ± 0.6 vs. 3.9 ± 0.4 species/m²; *df* = 1, 38; *t* = 11.40; *P* < 0.0001; Fig. 1). Although not statistically testable, the total number of species recorded at Moctezuma in Mexico was 45 species, with 34 species occurring under *A. adenophora* and 35 species occurring in the open—a mean difference in richness of 3%. At this site 11 species were restricted to the open and 9 species were restricted to under *A. adenophora* canopies. At Querétaro there were 38 species, with 30 species found in the open and 26 species under *A. adenophora*—a mean difference in richness of 13%. As at Moctezuma, 11 species were restricted to the open and 9 species were restricted to *A. adenophora* canopies. In China there were 111 species at Xujiaba, with 98 of these species found in the open away from *A. adenophora* but only 54 species beneath *A. adenophora*—a mean difference in total species richness of 54%. Fifty-five species were found only in vegetation away from *A. adenophora* canopies, and 9 species were found only under *A. adenophora*. There were 91 species in total at Taizhong in China and 79 species were found in the open; however, only 35 species were found under *A. adenophora*—a mean difference in richness of 56%. Fifty-eight species were found only in vegetation away from *A. adenophora* canopies, and 6 species were found only under *A. adenophora*. In India there were 56 species found at the Mussoorie sites 1 and 2 combined, and 25 species were found in the open and 15 species under *A. adenophora* canopies, a mean difference of 40% in total species richness.

Biogeographical differences in total percent cover between Mexico and China (cover was not measured in India) were even more striking. The total cover of other species beneath *A. adenophora* canopies in Mexico was 71% higher than in the open ($44.8\% \pm 2.6\%$ vs. $76.8\% \pm 6.2\%$ [means \pm SE]; *t* test, *df* = 1, 38; *t* = 4.78; *P* < 0.001). But in China *A. adenophora* canopies were correlated with 75% less total cover at Xujiaba ($102.6\% \pm 6.3\%$ vs. $25.5\% \pm 3.8\%$; *df* = 1, 38; *t* = 10.46; *P* < 0.001), and 79% less total cover at Taizhong ($83.3\% \pm 6.9\%$ vs. $17.2\% \pm 3.6\%$; *df* = 1, 38; *t* = 8.44; *P* < 0.001), suggesting strong inhibition. Note that adding cover of each species without relativizing as we did can lead to cover values greater than 100%. For total cover values less than 100%, the remaining nonvegetated cover

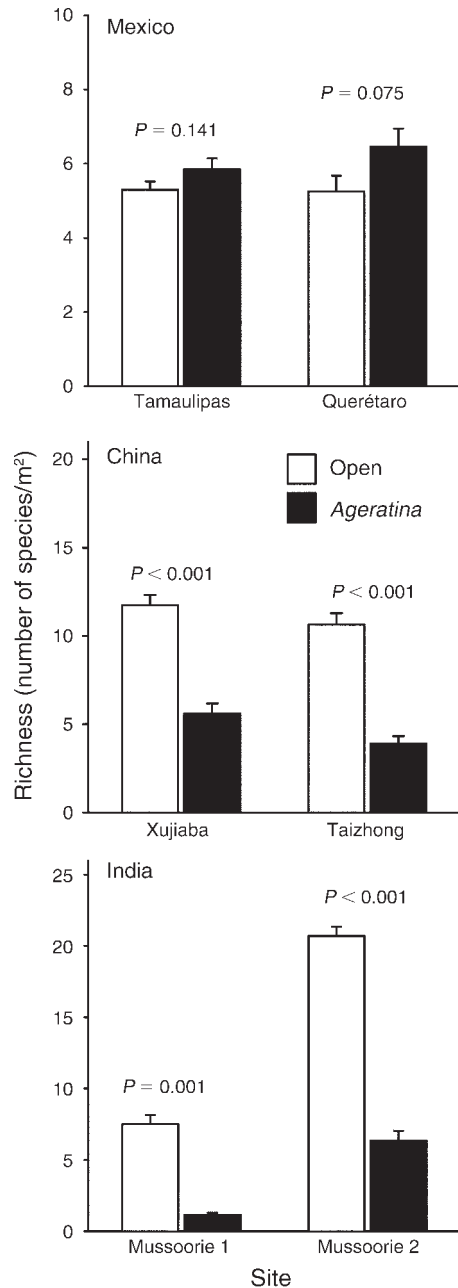


FIG. 1. Quadrat-based plant species richness under canopies of the subtropical invasive plant *Ageratina adenophora* and in the open at sites in Mexico, India, and China. Data are means \pm SE; *P* values above paired bars are from independent *t* tests.

was either bare ground or *A. adenophora* litter. The contribution of *A. adenophora* to total cover in its understories was less than 1% in both the native and nonnative ranges.

Volatiles of A. adenophora leaf litter inhibit the growth of other species

In petri dish experiments, *Bambusa arundinacea* seed germination was not inhibited (*t* = 0.180, *P* = 0.858) after

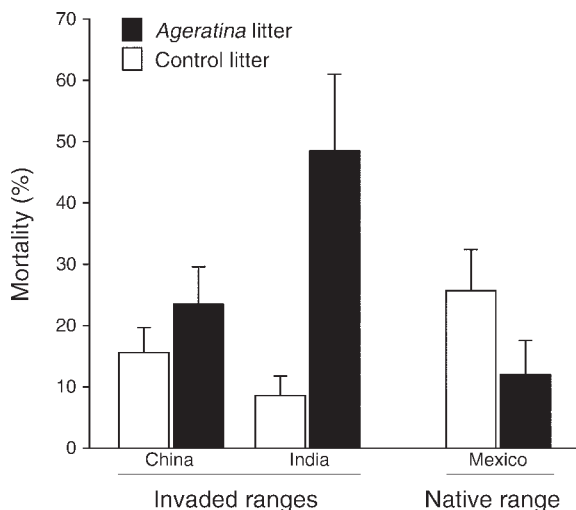


FIG. 2. Effects of volatiles from leaf litter of *Ageratina adenophora*, relative to control litter, on the mortality of species native to its invaded (China or India) ranges and species from its native (Mexico) range. Data are means \pm SE; for ANOVA results see *Results: Volatile effects on species*....

7 d by *A. adenophora* VOCs, but the germination of *Bidens biternata* seeds was highly suppressed (for final percentages; $t = 6.767$, $P < 0.0001$; Appendix A). The growth of the roots and shoots of both *B. arundinacea* ($t_{rt} = 15.232$, $P < 0.0001$; $t_{sh} = 8.224$, $P < 0.0001$) and *B. biternata* ($t_{rt} = 9.329$, $P < 0.0001$; $t_{sh} = 14.109$, $P < 0.0001$) was significantly suppressed by VOCs from *A. adenophora* leaf litter (Appendix A).

Volatile effects on species from native and nonnative ranges

The germination or growth of some individual species showed significant responses, either positive or negative, to VOCs emitted from *A. adenophora* litter, but exposure to VOCs had no significant overall effect on the germination or biomass of the combined pool of species from any of the three regions (data not shown). Of the seven species native to China that germinated at rates high enough to allow comparisons of mortality, *Amaranthus tricolor* ($41.3\% \pm 11.4\%$ [mean \pm SE] mortality with *A. adenophora* litter VOCs vs. $14.8\% \pm 2.6\%$ in control) and *Oxalis comiculata* ($34.7\% \pm 6.6\%$ vs. $9.9\% \pm 5.4\%$) showed significant negative effects of VOCs as determined by pairwise t tests using the mean mortality in a bin as replicates. For the remaining five Chinese species, *Peperomia tetraphylla*, *Hemiphragma heterophyllum*, *Eupatorium chinense*, *Eupatorium japonicum*, and *Cucubalus baccifer*, there were no significant effects of VOCs from litter. Of the seven species native to India with germination rates high enough for comparisons, *Cassia fistula* ($66.7\% \pm 8.9\%$ vs. $16.7\% \pm 8.8\%$), *Bombax ceiba* ($50.8\% \pm 21.0\%$ vs. $13.6\% \pm 4.2\%$), and *Dillenia indica* (100% vs. 0%) showed significant effects of *A. adenophora* VOCs. VOCs had no effect on *Lowsonia alba*, *Bambusa arundinacea*, or

Withania somnifera. Of the five species native to Mexico with high enough germination rates, *A. adenophora* VOCs had no significant inhibitory effects on any species, and were correlated with significantly lower mortality for *Desmodium graham* (0% vs. $38\% \pm 11.5\%$ in the control). There were no significant effects of *A. adenophora* VOCs on *Viguiera* spp., *Poa* spp., *Panicum* spp., and *Senecio* spp. In a two-way ANOVA with region as a fixed factor (combining species from both invaded regions), bin as a fixed factor, and with the mean for each species as a single replicate, the region \times treatment interaction was significant ($F = 5.201$; $df = 1, 37$; $P = 0.029$; Fig. 2), indicating that VOCs from *A. adenophora* litter had more inhibitory effects on species from the nonnative range of the invader than the native range.

Concentrations of some volatiles differ between native and invasive ranges

Many monoterpenes and sesquiterpenes were identified in the headspace analysis of *A. adenophora* leaf litter produced by plants raised from seeds collected from Mexico, China, and India (Appendices B and C). Plants from the native Mexican populations emitted significantly different concentrations of six different VOCs than either the invasive Chinese and Indian populations (Fig. 3; Appendices C and D). Plants from the Chinese and Indian populations showed very similar patterns in the VOCs that were emitted. Two monoterpenes, 2-carene and α -phellandrene, were at higher concentrations in the headspace of litter of plants from the invasive ranges; whereas the monoterpene β -pinene, and the three sesquiterpenes (*E*)- β -caryophyllene, (*E*)- α -bergamotene, and bicyclogermacrene, were at higher levels in the headspace of litter from plants of the native range. Direct extraction of litter from plants of the native range revealed the same terpenes in the same proportions as found in the headspace analyses, but recorded higher concentrations of the larger compounds (Appendix E).

DISCUSSION

Ageratina adenophora, an invasive plant throughout the subtropics, was correlated with higher diversity and greater abundances of understory plants in its native range in Mexico. In contrast, in its nonnative ranges in China and India the presence of *A. adenophora* shrubs correlated with striking decreases in the richness and cover of native species. Although our limited number of field sites warrants caution in extrapolating to the whole of its invasive range, other studies of *A. adenophora* in Asia have shown negative effects on native species that are similar to those presented here. Based on regression relationships at the scale of 25-m² plots in southwest China, Lu and Ma (2005) found that native species richness averaged >25 species per plot when *A. adenophora* cover was near zero; however, when *A. adenophora* cover reached $\sim 60\%$, native species richness

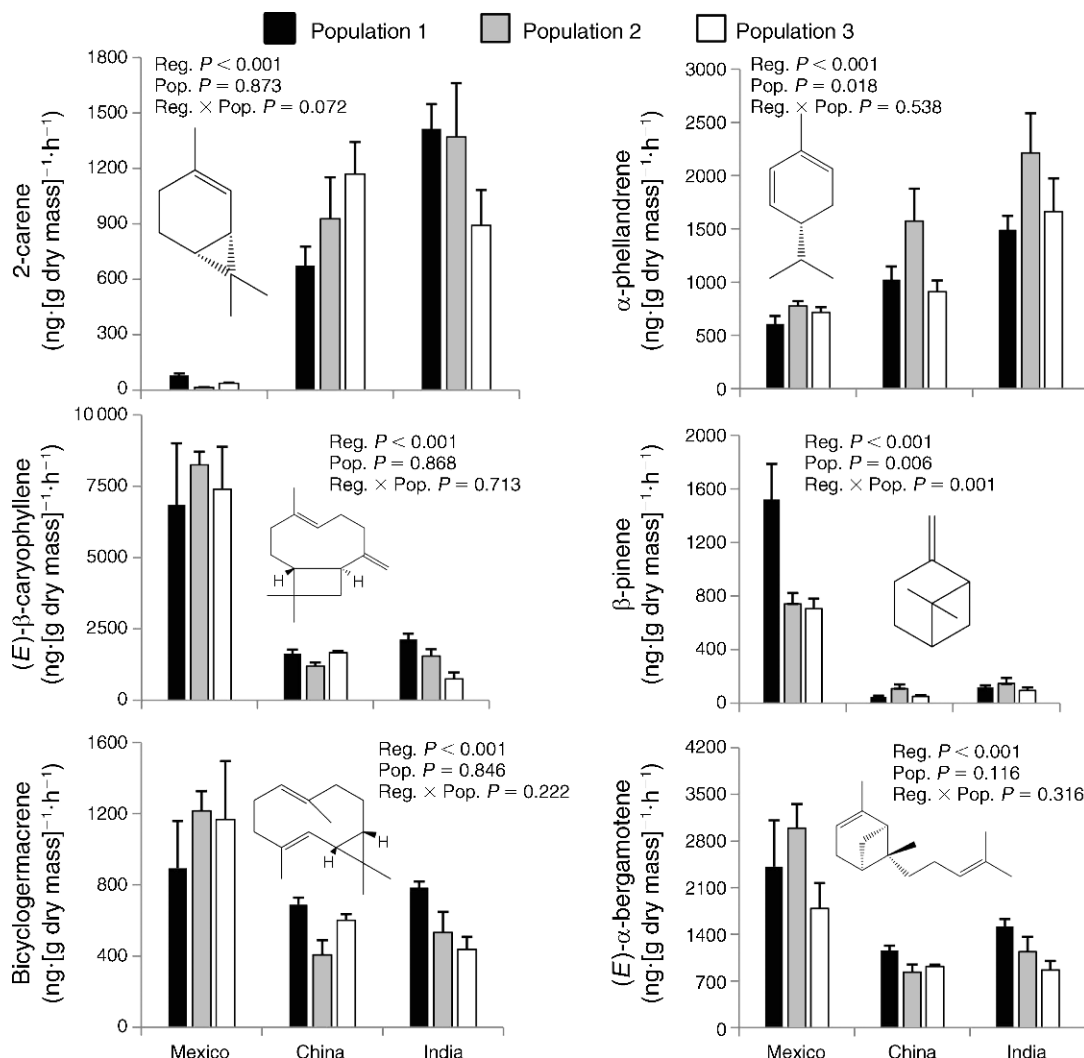


FIG. 3. Biogeographical differences in the emission of three monoterpenes [β -pinene, 2-carene, and α -phellandrene] and three sesquiterpenes [(E)- β -caryophyllene, (E)- α -bergamotene, and bicyclogermacrene]. Data are means + SE; P values are shown for two-way ANOVA.

declined to <5 species per plot. To our knowledge, no other study has quantitatively estimated the impact of an invasive plant species in the field in both its native and nonnative ranges, but a number of studies have clearly documented strong negative impacts of many invaders in their nonnative ranges (e.g., Hejda et al. 2009).

Many factors are likely to contribute to the invasive success of *A. adenophora*, but our findings suggest the potential of allelopathic VOCs as one of these. The allelopathic effects of VOCs from the litter of *A. adenophora* were stronger on species from the nonnative range than from the native range, a finding consistent with the novel-weapons hypothesis (NWH; Callaway and Ridenour 2004). Similarly, Ens et al. (2009a) examined VOCs produced by the roots of the invasive *Chrysanthemoides monilifera* in Australia and detected

three volatile compounds that were found exclusively in *C. monilifera* roots and its rhizosphere. Higher production of terpenes was observed in *C. monilifera* roots, while *Acacia longifera*, a native species, produced more alkanes. Extracts from leaves and roots of *C. monilifera* suppressed more native Australian species than extracts from the dominant native *Acacia* (Ens et al. 2009b). That study, however, lacked biogeographical comparisons of VOCs in native and invaded ranges.

Here, we have shown that production of a volatile compound, to which plant species growing in invaded regions appear to be more susceptible, has the potential to contribute to the invasive success of *A. adenophora*. Of course, this is unlikely to fully explain *A. adenophora* invasion and does not rule out the importance of other mechanisms. For example, Niu et al. (2007) found that *A. adenophora* and soil biota in the invaded range

interacted to establish positive feedbacks on *A. adenophora*, and that *A. adenophora* modified soil communities in ways that negatively impacted other species in the invasive range, suggesting the possibility that escape from soil pathogens may be important for invasion. In China, field surveys found an absence of specialist herbivores on *A. adenophora* (with the exception of a galling insect, *Procecidochares utilis* Stone, which was introduced into China in 1984), and that virtually no native generalists attack the plant (Feng et al. 2009). *Procecidochares utilis* was introduced as a biological control agent in India in 1963, but has been reported to cause only minor damage (Jayanth 2000). As for any exotic invasion, the relative importance of different mechanisms must be disentangled if we are to fully explain the process.

So far, the NWH has been explored in the context of regional novelty; i.e., the idea that some secondary metabolites might be unusually potent as allelopathic, antibiotic, or defense agents because of their novelty to organisms in nonnative ranges. However, it was not proposed that the invasive species would produce different concentrations of compounds in its nonnative ranges, as we have demonstrated here (but see Wikström et al. 2006, Ridenour et al. 2008). Our study suggests two potentially different interpretations of biochemical novelty in *A. adenophora* invasion, but our results do not favor one interpretation over the other. First, the effects in this experiment may be due to the adaptive naiveté of species from China and Mexico to chemicals produced by *A. adenophora*, no matter what the biogeographical source of the invader; the basic NWH. But we also found that the concentrations of some VOCs from leaf litter from populations of *A. adenophora* from China and India (grown under common conditions) were markedly different than those emitted from the leaf litter of *A. adenophora* grown from seed collected from Mexican plants, although many VOC concentrations were similar. Thus the biogeographical effects reported here might be due to the particular concentrations of VOCs produced by plants in invasive populations. However, there were no chemicals that were found only in either the native or nonnative ranges. The novelty of VOCs in nonnative ranges or novel concentrations of compounds are also conceptually consistent with ideas of the importance of evolving biochemical novelty in arms races between plants and their herbivore enemies (Ehrlich and Raven 1966).

The concentrations of 2-carene in the VOCs of leaf litter produced by *A. adenophora* plants from the invaded range (grown in a common garden) were three orders of magnitude higher than concentrations in VOCs from litter produced by *A. adenophora* plants from the native range (Fig. 3). α -Phellandrene emission was about twice as high for populations from the invaded ranges as for populations from the native range. 2-Carene has been reported to have allelopathic effects (Vokou et al. 2003), and Kpoviessi et al. (2009) found

that α -phellandrene, a constituent of the essential oil of *Justicia anselliana*, had allelopathic effects. If these compounds contribute to the allelopathic effects of *A. adenophora* VOCs, high concentrations may have been selected for in the nonnative ranges (Callaway and Ridenour 2004, Wikström et al. 2006, Ridenour et al. 2008). However, β -pinene and (*E*)- β -caryophyllene were emitted in far higher amounts from litter from Mexican plants than from the litter of Indian or Chinese plants and these chemicals have also been reported to have important defense properties (Köllner et al. 2008). Perhaps these compounds are less active as allelopathic agents than 2-carene and α -phellandrene. Or, perhaps they are important in defense against specialist enemies that should be less abundant in the invaded ranges (Feng et al. 2009).

Our finding that all populations from India and China differed from all populations from Mexico raises the possibility that invasive populations of *A. adenophora* experience generally consistent selection to produce higher levels of some biologically effective compounds in their nonnative ranges. However, our population-scale sampling was far too small to exclude founder effects, an equally valid interpretation of differences in the amounts of specific VOCs produced in the native and nonnative ranges. Regardless, our results add to an emerging body of work emphasizing the biological importance of VOCs emitted from plants as allelopathic agents (Kessler and Baldwin 2002, Karban 2007). However, our results could be strengthened by comparisons of *A. adenophora* litter to litter from native plants in the invader's nonnative range in both pots and Petri dish experiments. Also, Inderjit et al. (2009) suggested the use of "silenced" plants to acquire conclusive evidence that the release of chemicals does matter in plant-plant interactions, but silencing a perennial plant for the production of more than one chemical may be a significant challenge and performing field experiments with silenced plants is yet another challenge. Perhaps the best way to demonstrate the role of VOCs produced by *A. adenophora* VOCs in the field will be through manipulative field studies that discriminate the effects of VOCs from those of competitors, pathogens, and herbivores.

Plants release chemicals into their immediate environments to forage for resources, defend themselves against consumers, communicate with each other, nurture mutualists, and to gain advantages over competitors (Inderjit et al. 2006, Karban 2007, Metlen et al. 2009, Pollock et al. 2009). Our spatially and regionally explicit results add to the body of knowledge about allelochemicals by suggesting the possibility of regional evolutionary trajectories (Thompson 2005) and have several interesting implications for community ecology. First, they add to a growing body of literature indicating that there is some degree of species specificity in the effects of plant-released secondary metabolites in plant-plant interactions (see Callaway 1998, Callaway et

al. 2005). Second, if species-specific interactions among plants drive natural selection, then assemblages of plants, or communities, may be less individualistic than previously thought (see Lortie et al. 2004). Third, regional evolutionary trajectories suggest that novel competitive mechanisms used by invaders may disrupt coevolved interactions among long-associated native species. Finally, our results are consistent with the NWH, the idea that some invaders may succeed because they possess biologically active biochemicals to which species in the nonnative range have not adapted.

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APPENDIX A

Figures summarizing seedling length and germination data of *Bidens biternata* and *Bambusa arundinacea* grown in the presence or absence of *Ageratina adenophora* leaf litter (*Ecological Archives* E092-028-A1).

APPENDIX B

A figure presenting volatiles collected from headspace of 200-mg air-dried leaf material of three different origins (*Ecological Archives* E092-028-A2).

APPENDIX C

A table presenting the volatile compounds collected from headspace of leaf litter of *Ageratina adenophora* of different regional origins (*Ecological Archives* E092-028-A3).

APPENDIX D

Tables for two-way ANOVAs for the effect of region (Mexico, China, or India) and population on the concentration of six selected terpenes from *Ageratina adenophora* (*Ecological Archives* E092-028-A4).

APPENDIX E

A figure comparing volatiles collected through solvent extraction or from headspace of 200-mg air-dried leaf material of three different origins (*Ecological Archives* E092-028-A5).