

# Enhanced allelopathy and competitive ability of invasive plant *Solidago canadensis* in its introduced range

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## Abstract

### Aims

Why invasive plants are more competitive in their introduced range than native range is still an unanswered question in plant invasion ecology. Here, we used the model invasive plant *Solidago canadensis* to test a hypothesis that enhanced production of allelopathic compounds results in greater competitive ability of invasive plants in the invaded range rather than in the native range. We also examined the degree to which the allelopathy contributes increased competitive ability of *S. canadensis* in the invaded range.

### Methods

We compared allelochemical production by *S. canadensis* growing in its native area (the USA) and invaded area (China) and also by populations that were collected from the two countries and grown together in a ‘common garden’ greenhouse experiment. We also tested the allelopathic effects of *S. canadensis* collected from either the USA or China on the germination of *Kummerowia striata* (a native plant in China). Finally, we conducted a common garden, greenhouse experiment in which *K. striata* was grown in monoculture or with *S. canadensis* from the USA or China to test the effects of allelopathy on plant–plant competition with suitable controls such

as adding activated carbon to the soil to absorb the allelochemicals and thereby eliminating any corresponding allelopathic effects.

### Important findings

Allelochemical contents (total phenolics, total flavones and total saponins) and allelopathic effects were greater in *S. canadensis* sampled from China than those from the USA as demonstrated in a field survey and a common garden experiment. Inhibition of *K. striata* germination using *S. canadensis* extracts or previously grown in soil was greater using samples from China than from the USA. The competitive ability of *S. canadensis* against *K. striata* was also greater for plants originating from China than those from the USA. Allelopathy could explain about 46% of the difference. These findings demonstrated that *S. canadensis* has evolved to be more allelopathic and competitive in the introduced range and that allelopathy significantly contributes to increased competitiveness for this invasive species.

**Keywords:** allelopathy • biogeographical approach • common garden experiment • competition • invasion species

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## INTRODUCTION

Why invasive plants are often more competitive in their introduced range than in their native range has been a central

question in understanding plant invasive biology (Blossey and Nötzold 1995; Siemann and Rogers 2001; Siemann and Rogers 2003a; Wendy *et al.* 2008). Researchers have offered various hypotheses to explain the increased competitive ability of

invasive plant species (Elton 1958; Blossey and Nötzold 1995; Callaway and Aschehoug 2000; Stockwell *et al.* 2003), and these include phenotypic plasticity (Thompson *et al.* 1991a; Thompson *et al.* 1991b; Thompson *et al.* 1991c; Williams *et al.* 1995), release from natural enemies (Elton 1958; Maron *et al.* 2004), evolution of increased competitive ability (Blossey and Nötzold 1995; Stockwell *et al.* 2003) and the production of allelopathic compounds (Callaway and Aschehoug 2000; Bais *et al.* 2003).

Phenotypic plasticity of invasive plants has traditionally been considered a key factor in helping invasive plants to adapt to novel environments and compete against native plants in recipient communities. For example, phenotypic plasticity enabled the exotic plant species *Spartina anglica* to invade and rapidly colonize new areas (Thompson *et al.* 1991a, Thompson *et al.* 1991b, Thompson *et al.* 1991c). Similarly, the exotic plant *Pennisetum setaceum* exhibited pronounced morphological variation in different habitats, allowing itself to become dominant across diverse habitats in the introduced range (Williams *et al.* 1995).

The success of some invasive plants in the new range may depend on the absence of natural enemies, which allows these plants to reallocate energy and resources from producing 'defensive weapons' towards growth (Blossey and Nötzold 1995). Thus, in the new range, exotic plants may have evolved a reduction in defense and an increase in growth or reproduction that may enhance their competitive ability (Blossey and Nötzold 1995). Evidence for increased size and growth in invasive species is common (Siemann and Rogers 2001, Siemann and Rogers 2003a; Siemann and Rogers 2003b; Wolfe 2002; Jakobs *et al.* 2004; Müller and Martens 2005). Experiments have also demonstrated that about half of the reported invasive species have greater competitive ability in the introduced range than in the original range (Bossdorf *et al.* 2005). However, the trade-off between defense and growth of invasive plants remains unclear. While Ridenour *et al.* (2008) reported that the invasive species *Centaurea maculosa* did not reduce investments in defense while evolving competitive traits during its invasion, Feng *et al.* (2009) found that the invasive species *Ageratina adenophora* in China evolved a reduced allocation to cell walls (which should reduce defense) and an increased allocation of N to photosynthesis (which should increase growth).

Allelopathy refers to the effects of one plant on another plant or organisms through the release of chemicals into the environment (Muller 1969; Callaway 2002; Bais *et al.* 2003; Hierro *et al.* 2003). Allelopathy has both defensive and competitive characteristics in many invasive plants (Hubbell *et al.* 1983; Watling *et al.* 2011; Mallik and Pellissier 2000; Bais *et al.* 2003; Callaway and Ridenour 2004; Prati and Bossdorf 2004; Stinson *et al.* 2006; Jarchow *et al.* 2009). Some invasive plants exude allelochemicals into the soil to both inhibit soilborne pathogens and defend against disease (Kumar *et al.* 2010; Wu *et al.* 2010; Zhang *et al.* 2009, Zhang *et al.* 2011; Mitrovic *et al.* 2012); others produce allelochemicals to repel

insects (Farooq *et al.* 2011; Glinwood *et al.* 2011; Watling *et al.* 2011). Allelopathy can also mediate competitive interactions between plants (Jarchow *et al.* 2009; Bais *et al.* 2003; Callaway and Ridenour 2004; Prati and Bossdorf 2004; Stinson *et al.* 2006). Experiments have indicated that chemicals released by some invasive plant species are more active against native plant species in the introduced range than against co-evolved species in the native range, which has suggested the 'novel weapons hypothesis' or 'allelopathic advantage against resident species hypothesis' (Rabotnov *et al.* 1982; Hierro and Callaway 2003; Cappuccino and Arnason 2006) and which helps explain why some invasive species are able to outcompete native plants in the introduced plant community. For example, the allelochemical 8-hydroxyquinoline released by *Centaurea diffusa*, (Vivanco *et al.* 2004), (±)-catechin released by *Centaurea maculosa* (Callaway and Aschehoug 2000) and 7,8-benzoflavone released by *Acroptilon repens* (Alford *et al.* 2007) had allelopathic effects on the native plants in the introduced ranges. When allelochemicals enhance the competitive ability of invaders against native plants, they might maintain or even enhance the production of allelopathic compounds in the new range (Bossdorf *et al.* 2005; Ridenour *et al.* 2008).

In the current research, using *Solidago canadensis* L. (goldenrod) as a model invasive plant, we tested the hypothesis that an invasive plant may enhance its production of allelopathic compounds and allelopathic effects and thereby enhancing its competitive ability in the invaded land.

*Solidago canadensis*, originating from North America (Weber 1997), is an invasive weed of southeastern China (Dong *et al.* 2006). *Solidago canadensis* has a strong allelopathic effect on local plants of China (Yang *et al.* 2007; Abhilasha *et al.* 2008). We compared the contents of three major allelochemicals (total flavones, total phenolics and total saponins, Zhang *et al.* 2011), the allelopathic effects and the competitive effects of *S. canadensis* collected from the native area (the USA) and from the invaded area (China) by employing a comparative biogeographical approach (Hierro *et al.* 2005) that included a field survey and experiments including common garden experiments.

## METHODS

The perennial herb *S. canadensis* forms large colonies that reduce the abundance of native vegetation in southern China. Although rapid growth and prolific reproduction (both sexual and asexual) clearly contribute to its successful invasion, its strong allelopathic effects on native plants (Yang *et al.* 2007), arbuscular mycorrhizal fungi that form symbioses with native plants (Zhang *et al.* 2007) and soilborne pathogens (Zhang *et al.* 2009; Zhang *et al.* 2011) may also contribute to its invasiveness. Here, we determined whether allelopathy of *S. canadensis* is greater in the introduced range than in the native range (field survey and Experiments 1 and 2) and the degree to which enhanced allelopathy affects its competitive ability (Experiment 3).

### Field survey: Growth and production of allelopathic chemical by *S. canadensis* in its native and introduced range

We conducted a field survey to test whether allelopathic compound production by *S. canadensis* is greater in the introduced area (China) than in the native area (the USA). We sampled 14 populations from North Carolina, USA and six populations from Zhejiang Province, China (Table S1). Average monthly temperature and precipitation during the growth period of *S. canadensis* (from March to November) in sampling areas from two countries were similar (Fig. S1). Soil conditions of sampling sites are shown in Table S1. Samples were carefully identified taxonomically based on four morphological characteristics that included basal leaves, triple-nerved leaves, stem and inflorescence as described by Patricia et al. (1980).

Shoot height and population density were measured in a 1 × 1-m area in each sampling site. Ten plants at each sampling site were collected. Shoots, seeds, and roots of these 10 plants were separated. Shoots and roots were dried at 65°C until constant mass was achieved, and seeds from 20 individual plants per population were air dried and stored at 4°C until they were used for the experiments.

The crude extracts from both belowground and aboveground parts of *S. canadensis* were prepared as described by Zhang et al. (2009) and were used to determine the concentrations of total flavones, total phenolics and total saponins. Total flavones in the crude extracts were determined by the NaNO<sub>2</sub>-Al(NO<sub>3</sub>)<sub>3</sub>-NaOH colorimetric assay with rutin as the reference substance (Harborne 1973; Yang et al. 2007). Total phenolic acids were measured by the Folin-Ciocalteu assay with gallic acid as the reference substance (Harborne 1989a, Harborne 1989b). Total saponins were detected by using vanillin-HClO<sub>4</sub> as the chromogenic reagent (Liang et al. 2008). The extracts were then used in Experiment 1, as described in the next section.

### Experiment 1: Allelopathic effects of *S. canadensis* (collected from China vs. the USA) extracts on seed germination of a native plant

The effect of *S. canadensis* extracts collected in the field survey on seed germination was examined using sand culture as described by Zhang et al. (2009). Briefly, the crude extracts from *S. canadensis* collected in China and the USA were diluted with sterilized water to a concentration of 3.75% (w/v), and 40ml of the diluted extract was added to pots containing 150g of sterilized sand; the final concentration of extract in each pot was 1% (w/w). Thirty seeds of *Kummerowia striata* (Thunb.) Schindl., which is a native plant in China, were sown in each pot. Five replicate pots were used for each crude extract. All pots were kept in a growth chamber (Safe Experimental Instrument Company, Haishu, Ningbo, China) with a 16-h-light and 8-h-dark photoperiod, 18°C (night) and 22°C (day) temperature regime, and 90% relative humidity. After seeds were sown, Hoagland's nutrient solution was

added to maintain normal seedling growth. The frequencies of successful seed germination were recorded for 21 days. Germination rate was calculated by dividing the number of germinated seeds by the number of sown seeds and multiplying by 100.

Data pertaining to aboveground biomass, height and concentrations of allelochemicals (total flavones, total phenolics and total saponins) for plants in the survey as well as allelopathic effects (seed germination rate of *K. striata*) in Experiment 1 were first subjected to a homogeneity test and then to a nested ANOVA to determine sources of variation. We treated country (China vs. the USA) as a fixed effect and populations within each country as random effects. When ANOVAs were significant, mean values were compared by least significant difference (LSD) at the 5% significance level. SPSS (V.16.0) was used for all statistical analyses in this study.

### Experiment 2: Common garden, greenhouse experiment with plant populations collected from the USA and China: allelochemical production and allelopathic effects on seed germination

We conducted a greenhouse experiment in China to measure the differences in allelopathy caused by *S. canadensis* collected from the USA and China. As there was no significant difference of allelochemicals among the 14 US populations (total phenolics,  $P = 0.725$ ; total flavones,  $P = 0.246$ ; total saponins,  $P = 0.580$ ), and among the six populations in China (total phenolics,  $P = 0.18$ ; total flavones,  $P = 0.691$ ; total saponins,  $P = 0.114$ ), we chose three populations from the USA and three from China for this experiment. For China, we selected sample 1, 2 and 6 from three habitats (river side, road site and waste land, Table S1). For the USA, we selected sample 5, 6 and 7 also from three habitats (forest edge, rail site and waste land, Table S1). The experimental set up had a split-plot design, with country (the USA and China) as main plots and populations as sub-plots. There were eight replicates for each population, yielding a total of 48 (3 populations per country × 2 countries × 8 replicate) pots. The soil used in the experiment was collected from Cixi City, Zhejiang Province, China (30°18'N, 121°10'E), where *S. canadensis* has invaded. The soil had a pH of 5.7 (in a 2.5:1 suspension of KCl aqueous solution:soil) and contained 29.8g kg<sup>-1</sup> organic matter, 1.14g kg<sup>-1</sup> nitrogen and 5.7mg kg<sup>-1</sup> extractable phosphorus.

To ensure the consistency of plants in Experiment 2, the seeds were germinated and pre-cultured in a plastic mesh plate with vermiculite and peat in the greenhouse with natural light and temperature. When seedlings were 4 cm tall, they were transplanted into 750-cm<sup>3</sup> plastic pots containing 1 kg soil. The pots were arranged in a completely randomized split-plot design in the greenhouse and were irrigated with deionized water every day but were not fertilized. Six months after transplanting, the plants were harvested. Aboveground tissue for each plant was cut at the root-shoot junction and dried at 65°C until constant mass was achieved, and then biomass was

determined by weighing. Pots were placed at 4°C until the roots could be washed (within 1 week). Belowground tissue was harvested by washing the soil particles off the roots/rhizomes with water. Belowground tissue was dried at 65°C until constant mass was achieved. The same soil type was further used for a germination experiment as described below.

Crude extracts from aboveground and belowground tissues of *S. canadensis* were prepared as described in the field survey. The contents of allelochemicals (total flavones, total phenolics and total saponins) in crude extracts in *S. canadensis* (Experiment 2A) and the allelopathic effects on the germination of *K. striata* seeds in sand (Experiment 2B) were also tested as described in the field survey. In addition, the soil in the original pots was used in another germination test (Experiment 2C) as described in the next paragraph.

For Experiment 2C, the soil in each pot was divided into two parts, and activated carbon was mixed into one part but not the other. Activated carbon is able to absorb allelochemicals (Ridenour and Callaway 2001; Wurst and van Beersum 2009) and was used to create lower levels of allelochemicals in the experiment. Seeds of *K. striata* were planted in each subplot. This factorial experiment had two main effects—country (the USA vs. China) and activated carbon ( $\pm$ ). Germination was assessed as described for the Experiment 1.

Differences in the aboveground biomass, height and concentrations of allelochemicals (total flavones, total phenolics and total saponins) as well as in allelopathic effects (seed germination rate of *K. striata*) as affected by country of origin were analyzed using nested ANOVAs. All factors were considered as fixed effects except population (country), which was analyzed as a random effect. When allelopathic effects (seed germination rate of *K. striata*) within each country were compared for pots with and without activated carbon, a two-way ANOVA in the general liner model was used.

Germination rates were arcsine transformed to satisfy variance assumptions before ANOVAs were performed. When ANOVAs were significant, mean values were compared by LSD at the 5% significance level.

### Experiment 3: effects of allelopathy on competition

A common garden, greenhouse experiment (Experiment 3) was performed to determine whether enhanced allelopathy contributes to the competitive ability of *S. canadensis*. This experiment used the same populations from the USA and China that were used for Experiment 2. The forb *K. striata* was used as the native competitor. *K. striata* is a common weed in crop fields, orchards and abandoned land (Chen *et al.* 2004) and usually occurs in areas invaded by *S. canadensis*. In our preliminary three-year field observation in China, we found that *K. striata* was replaced gradually by *S. canadensis* in the *K. striata*-dominated weed communities (Zhang *et al.* 2011). Also, from our field surveys carried out in the USA, we found that *K. striata* and *S. canadensis* coexist in weed communities. Thus, we selected this legume as the reference native plant for this study.

The experiment had a split-plot design with country (the USA and China) as the main plots, activated carbon ( $\pm$ ) as the sub-plots, population as the sub-sub-plots and culture types (monocultures of invasive or native and mixtures of invasive and native) as the sub-sub-sub-plots. Five replicate microcosms for each treatment yielded a total of 130 microcosms. Each microcosm measured 20 × 15 × 20 cm (length × width × height) and contained 6 kg of soil. The soil was same as used in Experiment 2.

To ensure the initial consistency of plants in the experiment, the seeds were germinated and pre-cultured as described in the Experiment 2. Two 4-week-old seedlings were transplanted into each microcosm so that the microcosm would contain one of the following: two *S. canadensis* seedlings, two *K. striata* seedlings or one seedling from each species. For carbon treatment, finely ground activated carbon was mixed into the soil at a rate of 20 ml l<sup>-1</sup>.

Microcosms were arranged in a greenhouse in a completely randomized block design. Plants were watered daily. No additional nutrients were added. Experiment 3 was terminated 6 months after transplanting, which coincided with floral initiation. We separated the shoots from roots. All shoots were oven dried to obtain constant mass for the measurement of dry shoot biomass.

The aggressivity indices of plants (Scheublin *et al.* 2007; Zhang *et al.* 2010) were calculated using the shoot biomasses of *K. striata* and *S. canadensis* in monoculture and mixture. The aggressivity index is given as  $(Y_{ij}/Y_{ii}) - (Y_{ji}/Y_{jj})$ , where  $Y_{ij}$  and  $Y_{ii}$  are the shoot biomasses of *K. striata* in mixture and monoculture and  $Y_{ji}$  and  $Y_{jj}$  are the shoot biomasses of *S. canadensis* in mixture and monoculture, respectively. The lower the aggressivity index, the more competitive is *S. canadensis* compared with *K. striata*.

The allelopathic contribution to *S. canadensis*'s competitiveness was calculated as the difference between *K. striata* biomass in the '*S. canadensis* + *K. striata* with activated carbon' treatment and *K. striata* biomass in the '*S. canadensis* + *K. striata* without activated carbon' treatment divided by the difference between *K. striata* biomass in the '*K. striata* monoculture without activated carbon' treatment and *K. striata* biomass in the '*S. canadensis* + *K. striata* without activated carbon' treatment (Ridenour and Callaway 2001).

Aggressivity indices of *S. canadensis* (the USA or China) and the effects of activated carbon on biomass under monoculture or mixed culture were compared with two-way ANOVAs using the general liner model. The effect of activated carbon treatments on *K. striata* biomass was compared with a one-way ANOVA. We used a nested ANOVA when comparing the variation in biomass, aggressivity indices and allelopathic contributions of *S. canadensis* from the USA and China with or without activated carbon. The variation in *K. striata* biomass, when grown with *S. canadensis* from the USA or when grown with *S. canadensis* from China, was also compared using a nested ANOVA. All factors were considered as fixed effects except for the population (country) variable, which was analyzed as a random effect.



Allelopathic contributions were arcsine transformed to satisfy the variance assumptions before ANOVAs were performed. When ANOVAs were significant, mean values were compared by using the LSD at the 5% significance level.

## RESULTS

### Growth and allelopathy of *S. canadensis* in or from its non-native and native range (field survey and Experiments 1 and 2)

In the field surveys, plants in six non-native populations of *S. canadensis* from China were significantly taller and had significantly more biomass than the plants in the 14 native populations from the USA (for plant height,  $F_{1,14} = 50.77$ ,  $P = 0.000$ ; for biomass,  $F_{1,14} = 74.50$ ,  $P = 0.000$ ) (Fig. 1).

Results from the first common garden experiment also showed that China's *S. canadensis* plants were taller and had more biomass than *S. canadensis* plants from the USA (for plant height,  $F_{1,4} = 11.439$ ,  $P = 0.028$ ; for biomass,  $F_{1,4} = 12.735$ ,  $P = 0.024$ ) (Fig. 1).

In the field survey, concentrations of three main groups of allelochemicals (total phenolics, total flavones and total saponins) were greater in extracts isolated from *S. canadensis* plants grown in China than those grown in the USA ( $P < 0.05$ , Fig. 2a). The same was true when *S. canadensis* populations originating from China and the USA were grown under common garden, greenhouse conditions in Experiment 2A ( $P < 0.05$ , Fig. 2b).

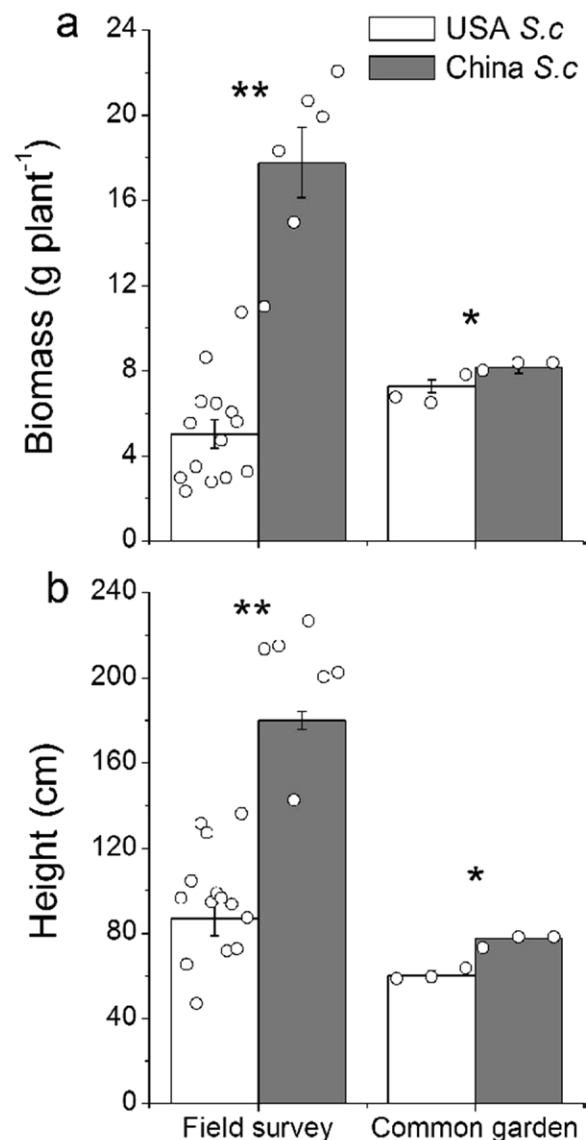
In Experiment 1, *K. striata* germination rates were lower in sand treated with extracts from *S. canadensis* grown in China, rather than those grown in the USA (for aboveground extracts,  $F_{1,14} = 11.563$ ,  $P = 0.028$ ; for belowground extracts,  $F_{1,14} = 12.367$ ,  $P = 0.023$ ) (Fig. 3a).

In Experiment 2B, germination rates of *K. striata* in sand were also lower when the sand was previously treated with extracts of greenhouse-grown *S. canadensis* of Chinese origin rather than those of US origin (for aboveground extracts,  $F_{1,4} = 10.243$ ,  $P = 0.037$ ; for belowground extracts,  $F_{1,4} = 9.287$ ,  $P = 0.039$ ) (Fig. 3b).

In the common garden Experiment 2C, *K. striata* germination was 13.8% lower in soil (from the Experiment 2) pre-grown with *S. canadensis* from China than that from the USA when receiving no activated carbon ( $P < 0.05$ , Fig. 4). However, the addition of activated carbon eliminated the differences in germination caused by *S. canadensis* from both China and the USA ( $P > 0.05$ , Fig. 4) and demonstrated increased germination rates ( $P < 0.05$ ).

### Effects of *S. canadensis* allelopathy on competition (Experiment 3)

Adding activated carbon to soil significantly reduced *S. canadensis* shoot biomass in monoculture whether the plant populations were from the USA ( $F_{1,21} = 16.078$ ,  $P = 0.001$ ) or China ( $F_{1,18} = 9.429$ ,  $P = 0.007$ ) but did not change the biomass in mixed culture with *K. striata* (for *S. canadensis* from

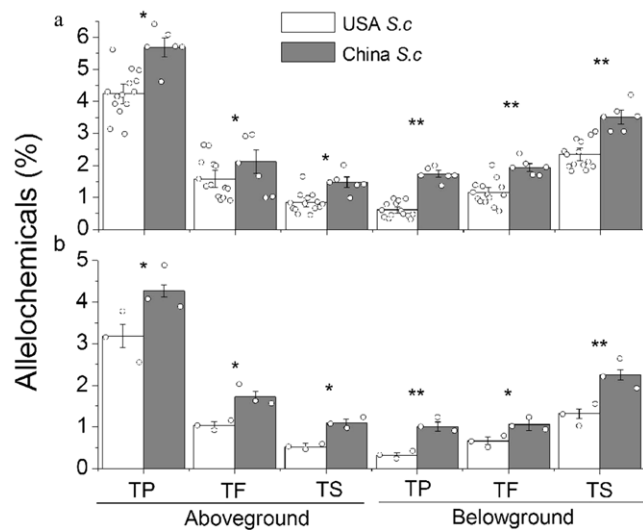


**Figure 1:** Plant height (a) and the aboveground biomass (b) of *S. canadensis* from either the USA or China in a field survey and a common garden experiment (Experiment 1). Values are mean values  $\pm$  SE; circles represent population mean values; \* indicates  $P < 0.05$ ; \*\* indicates  $P < 0.01$ .

the USA,  $F_{1,26} = 2.368$ ,  $P = 0.136$ ; for *S. canadensis* from China,  $F_{1,25} = 0.022$ ,  $P = 0.883$ ) (Fig. 5).

Biomass was always greater for *S. canadensis* from China than those originating from the USA either under monoculture (without activated carbon,  $F_{1,4} = 32.875$ ,  $P = 0.005$ ; with activated carbon,  $F_{1,4} = 8.677$ ,  $P = 0.042$ ) or under mixed culture (without activated carbon,  $F_{1,4} = 8.345$ ,  $P = 0.045$ ; with activated carbon,  $F_{1,4} = 5.373$ ,  $P = 0.082$ ) (Fig. 5).

Unlike the biomass of the invasive *S. canadensis* growing under monoculture, the biomass of native *K. striata* under monoculture was enhanced by activated carbon ( $F_{1,7} =$

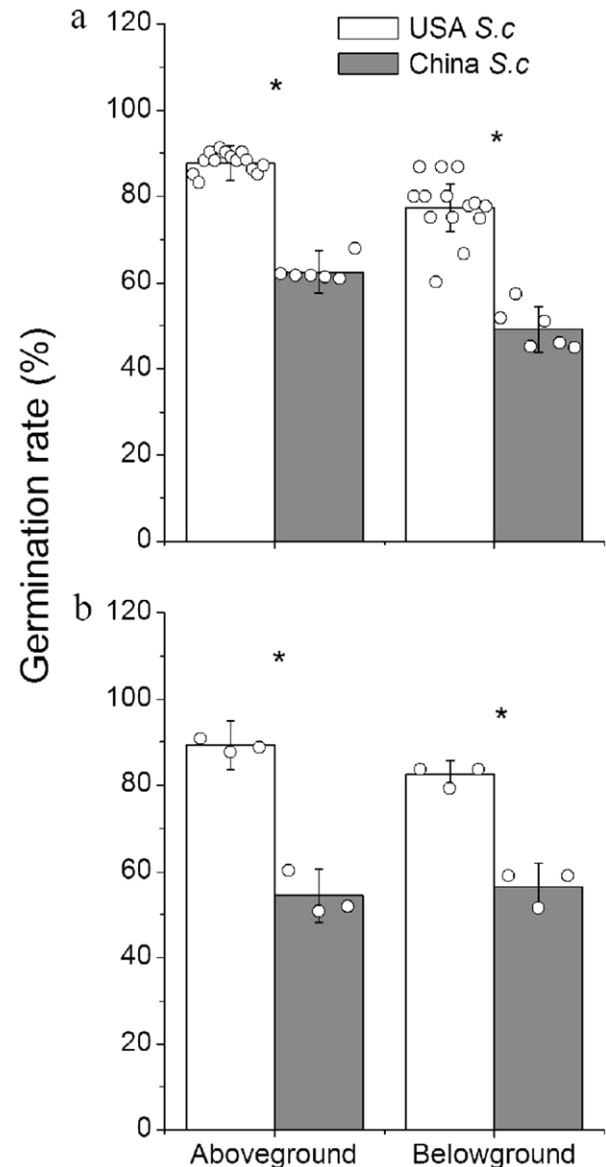


**Figure 2:** Concentrations of the three group allelochemicals (total phenolics, TP; total flavones, TF; total saponins, TS) in aboveground and belowground parts of *S. canadensis* from either the USA or China in the field survey (a) and common garden conditions in the Experiment 2A (b). Circles represent population mean values; values are mean values  $\pm$  SE; \* indicates  $P < 0.05$ ; \*\* indicates  $P < 0.01$ .

9.561,  $P = 0.018$ ) (Fig. 5). When grown in mixed culture with *S. canadensis* from China, *K. striata* biomass was enhanced by activated carbon ( $F_{1,23} = 4.737$ ,  $P = 0.040$ ) (Fig. 5). The activated carbon treatment, however, did not affect *K. striata* biomass when grown in mixed culture with *S. canadensis* from the USA ( $F_{1,26} = 1.031$ ,  $P = 0.319$ ) (Fig. 5).

In mixed culture, *K. striata* biomass differed depending on the origin of *S. canadensis*. Without the addition of activated carbon, *K. striata* biomass was lower with *S. canadensis* from China than with *S. canadensis* from the USA ( $F_{1,4} = 69.637$ ,  $P = 0.002$ ; Fig. 5). With the addition of activated carbon, however, *K. striata* biomass was similar regardless of the origin of *S. canadensis* plants ( $F_{1,4} = 0.055$ ,  $P = 0.825$ ) (Fig. 5).

*S. canadensis*'s competitive ability can be demonstrated using the aggressivity index, which was based on shoot biomass data. A higher competitive ability is illustrated by a more negative aggressivity index. Without adding activated carbon, the aggressivity index was lower (more negative) for *S. canadensis* from China than those from the USA ( $F_{1,12} = 17.146$ ,  $P = 0.001$ ) (Fig. 6), indicating that *S. canadensis* from China had greater competitive ability against *K. striata* than *S. canadensis* from the USA. With the addition of activated carbon, however, the aggressivity indices did not differ between *S. canadensis* from China and that from the USA ( $F_{1,4} = 0.049$ ,  $P = 0.836$ ) because activated carbon significantly increased the aggressivity index of *S. canadensis* from China ( $F_{1,10} = 7.353$ ,  $P = 0.022$ ) but not of *S. canadensis* from the USA ( $F_{1,22} = 0.035$ ,  $P = 0.854$ ) (Fig. 6). The latter finding indicates that addition of activated carbon reduced the competitive ability of *S. canadensis* from China.

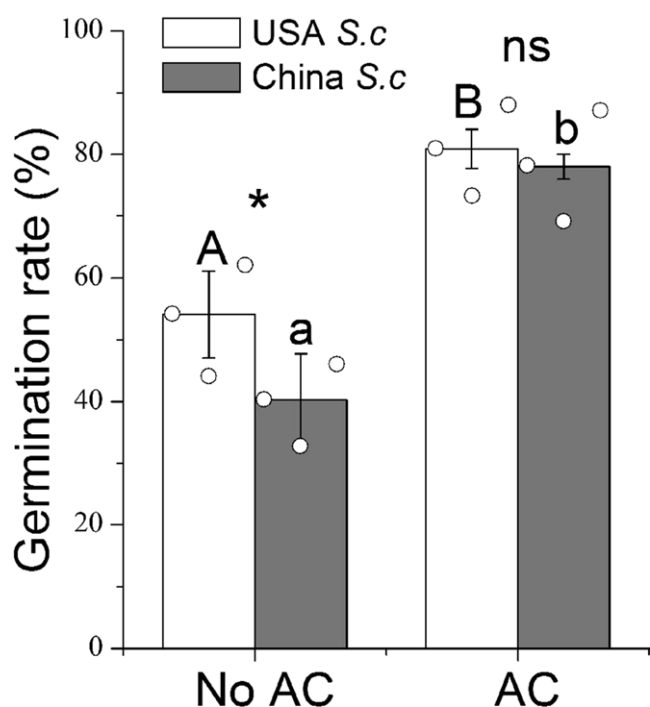


**Figure 3:** The germination rate of *K. striata* in sand as affected by aboveground and belowground extracts of *S. canadensis* obtained from either the USA or China in the field survey (a) and common garden conditions in Experiment 2B (b). Circles represent population mean values; values are mean values  $\pm$  SE; \* indicates  $P < 0.05$ .

In Experiment 3, the contribution of allelopathy to the competitive ability of *S. canadensis* against *K. striata* was greater for *S. canadensis* from China than for *S. canadensis* from the USA ( $P < 0.05$ ), whether the assessment was based on root biomass, shoot biomass, or total biomass (Fig. 7).

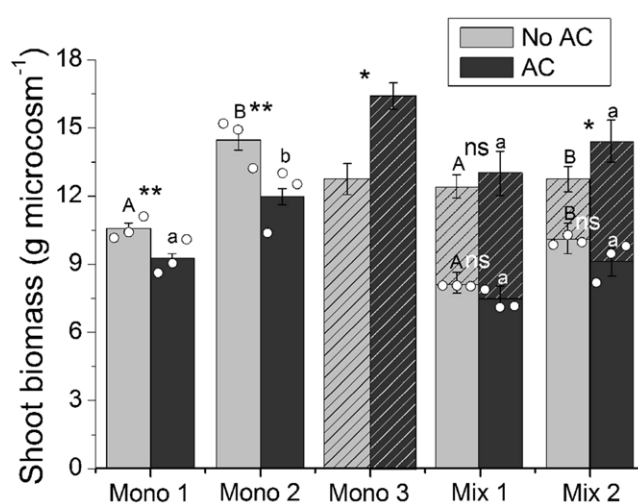
## DISCUSSION

Comparative biogeographical approach offers ample opportunities to study exotic plant invasions (Hierro *et al.* 2005). In our study, although *S. canadensis* has a number of congeners and



**Figure 4:** The germination rate of *K. striata* in soil (from Experiment 2) previously planted with *S. canadensis* obtained from either the USA or China that was supplemented or not supplemented with activated carbon (AC vs. No AC) in Experiment 2C. Circles represent population mean values; values are mean values  $\pm$  SE; \* indicates a significant difference between the effects of *S. canadensis* origin within each activated carbon treatment at  $P < 0.05$ ; for the soil that previously contained plants from either the USA or China, mean values with different uppercase or lowercase letters, respectively, are significantly different at  $P < 0.05$ ; ns indicates no significance.

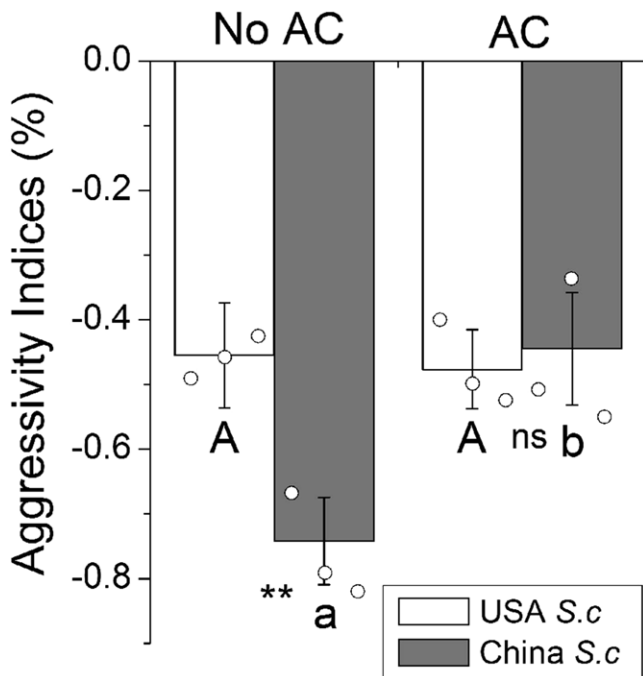
the taxonomy within the genus *Solidago* remains complex (Wu et al. 2005), we managed to find some unique characteristics in *S. canadensis* that are reliable and helped us to distinguish *S. canadensis* from congeners and some other similar species in the field (Patricia et al. 1980). In a previous experiment, we confirmed that the populations from USA and China were *S. canadensis* by employing careful morphological studies (Zhang 2011). Thus, in this study, we identified and collected the 'right' samples of *S. canadensis* in the field using four distinctive morphological characteristics (Patricia et al. 1980; Zhang 2011). Our field survey indicated that *S. canadensis* grows larger in the introduced range than in the native range, and the first common garden experiment provided some evidence to support the genetic basis of the observed growth difference. Other studies have shown that most invasive plant species either evolve a more rapid growth rate or exhibit greater phenotypic plasticity in the new habitats (Mooney and Cleland 2001; Carroll et al. 2005; Sax et al. 2007; Prentis et al. 2008). Invasive Chinese tallow trees, for example, are larger in size in the introduced range than within their native range (Siemann and Rogers 2001), and the invasive plant *Lepidium draba* also has greater shoot and root biomass and numbers of ramets in the introduced range than in



**Figure 5:** Shoot biomass of mono-*S. canadensis* from the USA (Mono 1) and from China (Mono 2), mono-*K. striata* (Mono 3), and their mixtures (Mix 1 and Mix 2) with or without activated carbon (AC or No AC) in the Experiment 3. Diagonal lines indicate the shoot biomass of *K. striata*. Circles represent population mean values of *S. canadensis*. Within each of the five treatments, \* or \*\* indicates a significant difference between AC and No AC at  $P < 0.05$  or  $P < 0.01$ , respectively. In comparisons between Mono 1 and Mono 2 and between Mix 1 and Mix 2, different uppercase or lowercase letters indicate significant differences between the indicated mean values.

the native range (Müller and Martens 2005). As in the present study, the latter two studies included common garden experiments that indicated that these phenotypic differences had a probable genetic basis. These post-invasion, genetic-based differences are likely to help the invasive plants to compete against their native neighbors. In the case of the invasive plant *Solidago gigantea* (Asteraceae), the biomass is larger and the sexual and vegetative reproductive efforts were greater when growing in the invasive range than in the native range (Jakobs et al. 2001; Jakobs et al. 2004). Once established in an area, the rapid vegetative reproduction enables *S. gigantea* to compete strongly against its neighbors.

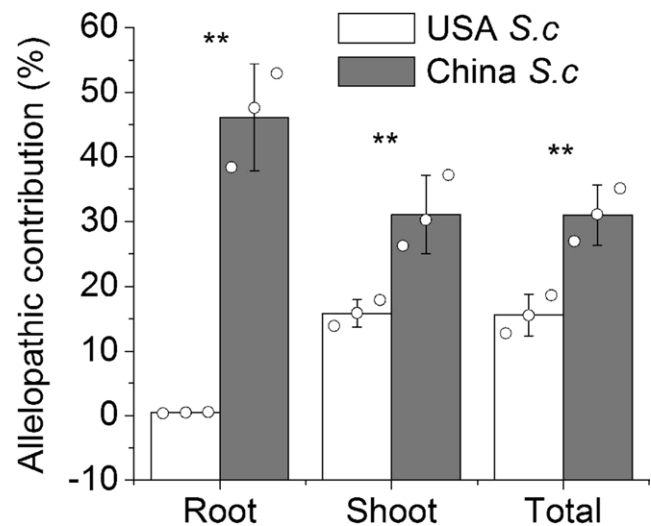
Our results from field survey and common garden experiment showed that the contents of the three kinds of allelopathic chemicals in *S. canadensis* were greater for plants in the invaded range than in the native range. These three chemicals (total flavones, total phenolics and total saponins) are the main allelochemicals produced by *S. canadensis* (Zhang et al. 2006; Mert-Türk 2006), which are known to play important roles in plant defense and allelopathic inhibition (Elliger et al. 1980; Inderjit 1996; Harborne and Williams 2000; Mert-Türk 2006; Kim and Lee 2011). For example, Kim and Lee (2011) found that the total phenolic compounds of invasive species reduced radicle growth of three native plant species by 60–80%. Our results also showed the allelopathic effects of *S. canadensis* on native plant seedlings were greater for plants in the invaded range than in the native range. These results implied that the three kinds of allelopathic chemicals may in



**Figure 6:** Competitive effects of *S. canadensis* from either the USA or China on *K. striata* with or without addition of activated carbon (AC or No AC) as indicated by aggressivity indices in the Experiment 3. Circles represent population mean values. Values are means  $\pm$  SE. Within the AC treatment or within the No AC treatment, \*\* indicates that the mean values differ at  $P < 0.01$  and ns indicates no significant difference. For treatments with *S. canadensis* from the USA, mean values with different uppercase letters are significantly different at the 5% level. For treatments with *S. canadensis* from China, mean values with different lowercase letters are significantly different at the 5% level.

part contributed to the observed allelopathy effects, although we do not rule out the plausible role(s) of some other chemicals. Our competition experiment further demonstrated that *S. canadensis* from China possessed a higher competitive ability than those from the USA, and that allelopathy contributed 46.1% to this enhanced competitive ability. Research results have been inconsistent about whether allelopathy contributes to competitive ability. Rich (2004) found no evidence that the invasive plant *Acer platanoides* used allelopathy to interfere with the growth of native plants, and the invasive plant *Centaurea maculosa* outperformed the native plant *Festuca idahoensis* in North America even when activated carbon was added to the soil (Ridenour and Callaway 2001). Inhibition of mycorrhizal fungi by *Alliaria petiolata* (garlic mustard), however, was greater in the invaded range than in the native range, and this resulted in greater inhibition in the invaded range where the native plants are dependent on the fungi (Callaway et al. 2008). In our study, the enhanced competitive ability of *S. canadensis* in the invaded land may be due to the enhanced allelochemical contents and allelopathic effects.

The mechanism(s) that whether allelopathy contributes to plant invasion remains unclear (Hubbell et al. 1983; Watling



**Figure 7:** Allelopathic contributions to the competitive ability of *S. canadensis* from the USA or China against the native *K. striata* in the Experiment 3. Circles represent population mean values. Values are means  $\pm$  SE. Within each category of plant biomass (root, shoot, or total), \*\* indicates a significant difference between *S. canadensis* from either the USA or China at  $P < 0.01$ .

et al. 2011; Mallik and Pellissier 2000; Bais et al. 2003; Callaway and Ridenour 2004; Prati and Bossdorf 2004; Stinson et al. 2006; Jarchow et al. 2009). Some studies suggested allelopathy could be a defensive strategy during plant invasion. Release of allelochemicals by the invasive shrub *Lonicera maackii*, for example, protects itself against attack by local amphibian larvae (Watling et al. 2011). Because of excreted secondary compounds, leaves of *Hymenaea courbaril* L. were not predated by the leaf-cutter ant *Atta cephalotes* L. (Hubbell et al. 1983). Similarly, *S. canadensis* reduces attack by the soilborne pathogens *Pythium ultimum* and *Rhizoctonia solani* through the exudation of allelochemicals (Zhang et al. 2009). Other studies suggested that allelopathy promotes plant invasion by increasing the competitive ability of invasive plants (Murrell et al. 2011; Rashid and Reshi 2012). An alien invasive species *Anthemis cotula*, for example, can excrete allelochemicals to inhibit seed germination and retarding seedling growth of its native competitors *Conyza canadensis* and *Galinsoga parviflora* (Rashid and Reshi 2012). In our current study, the *in planta* concentrations of the three kinds of allelochemicals and their allelopathic effects on seedlings of native plants were greater in the new location than in the original location, and as before, the first common garden experiment demonstrated that these differences probably had a genetic basis. Moreover, the competitive ability of *S. canadensis* in the new location was enhanced when compared with that in the original location, and allelopathy contributed 46.1% to the enhanced competitive ability. All these results implied that the allelopathic processes employed by *S. canadensis* expanded its population spread into the new range by enhancing the competitive ability of the species.



Allelopathic effects for the same species may somewhat differ between greenhouse and field experiments. For example, the allelopathic effects of *Malva sylvestris* and *Sisymbrium irio* on wheat and barley growth in greenhouse were different from those experiments carried out in the field (Qasem 2010). These differences may result from the greater complexity of plant interactions under field conditions. So far, however, few studies have investigated allelopathic effects of exotic plants in both the greenhouse and field (Hierro et al. 2005). In our holistic study, we studied allelopathy using field surveys and common garden experiments. Common garden experiments were conducted to determine whether differences in growth may have resulted from genetic factors. Overall, our results are consistent with the hypothesis that the invasive plant *S. canadensis* showed increased allelopathic potential, which increases its competitive ability in China and allowing it to colonize many new areas. Although we have no direct evidence that the increased allelopathic potential and competitive ability of *S. canadensis* in the invaded range results from recent genetic evolution for this species in the invaded range (it is possible that the populations in China represent highly competitive populations that were introduced from the USA or elsewhere), we suspected that the observed growth differences may be attributed, in part, resulting from rapid biological adaptations in the introduced range. Our study paved the way for future research about the population genetics of *S. canadensis* in both native and introduced ranges. Increased understanding of the evolution of invasive plants could help us predict, understand and better manage these invasions (Sax et al. 2007).

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