



# Allelopathic suppression by *Conyza canadensis* depends on the interaction between latitude and the degree of the plant's invasion

Congyan Wang<sup>1\*</sup>, Kun Jiang<sup>1</sup>, Jiawei Zhou<sup>1</sup> and Jun Liu<sup>1</sup>

Received: February 7, 2017

Accepted: March 3, 2017

## ABSTRACT

Allelopathic suppression of the growth of co-occurring plant species in invaded ecosystems is an important factor in some plant invasions. This study uses leaf extracts of the invasive plant species *Conyza canadensis* to determine its allelopathic effects along a latitudinal gradient, and under different cover classes and degrees of invasion, on seed germination and growth of *Lactuca sativa*, a sensitive bioindicator of allelochemicals. The allelopathic effects of *C. canadensis* on seedling height, root length, seedling biomass, germination percentage, germination potential, germination index, germination rate index, and vigor index of *L. sativa* increased significantly with increasing latitude. A possible explanation is that the leaves of plants growing in high latitudes secrete a higher concentration of allelochemicals than do leaves of plants growing in low latitudes. The allelopathic effects of *C. canadensis* on seedling height, seedling biomass, germination potential, germination index, germination rate index, and vigor index of *L. sativa* decreased with increasing degree of invasion. The more intense allelopathic effects of *C. canadensis* at lower degrees of invasion may enable it to establish populations in ecosystems by inhibiting the seed germination and growth of co-occurring species.

**Keywords:** allelopathic effects, *Conyza canadensis*, invasive plant species, *Lactuca sativa*, seed germination and growth

## Introduction

Plant invasions have inflicted serious damage on the structure and function of native ecosystems (Wang *et al.* 2016; 2017). Some plants successfully invade certain environments because they can release toxic chemicals and thereby pose an allelopathic effect on seed germination and growth of co-occurring species (Djurdjević *et al.* 2011; Hu & Zhang 2013; Wang *et al.* 2016). Plant invasions can occur in different degrees (i.e., different quantitative cover classes) in colonized ecosystems (Seastedt & Pyšek 2011; Wang *et al.*

2017). Meanwhile, the invasion of some plant species (such as *Conyza canadensis*) can occur across a latitudinal gradient (Yan *et al.* 2014). At present, the invasive plant species *C. canadensis* has spread into most regions of China (Weber *et al.* 2008; Hao *et al.* 2011; Yan *et al.* 2014). Seed germination and growth are highly important for plant recruitment and resource competition (Weiner *et al.* 1997; Turk & Tawaha 2003). The allelopathic effects of invasive plant species can decrease the competitiveness of co-occurring species. Thus, understanding the allelopathic effects of *C. canadensis* across its different invasion degrees along a latitudinal gradient on

<sup>1</sup> Institute of Environment and Ecology, Academy of Environmental Health and Ecological Security & School of the Environment and Safety Engineering, Jiangsu University, Zhenjiang 212013, P. R. China

\* Corresponding author: liuyuxue623@163.com

## Allelopathic suppression by *Conyza canadensis* depends on the interaction between latitude and the degree of the plant's invasion

seed germination and growth of co-occurring species may help us understand the mechanism of its successful invasion.

This study aims to understand the allelopathic effects of the invasive plant species *C. canadensis* on seed germination and growth of *Lactuca sativa*, taking into account invasion degree and latitude. The two plant species occur in the same ecosystem. The invasion degree of *C. canadensis* was evaluated based on its cover classes in the invaded sites. *Conyza canadensis* is a herbaceous annual invasive weed native to North America (mainly Canada) (Weaver 2001; Hao *et al.* 2011; Shah *et al.* 2014). It was first recorded in Yantai, Shandong Province of China in 1862 (Xie *et al.* 2001; Hao *et al.* 2011). *Conyza canadensis* has been listed as one of the most destructive and widespread invasive plant species in China because it poses a serious threat to the diversity and/or abundance of co-occurring plants (Weber *et al.* 2008; Hao *et al.* 2011). At present, this species has become a global invader (Zaplata 2011; Djurdjević *et al.* 2012; Shah *et al.* 2014). It thrives in many habitats, such as meadows, edges of forests, clearings, orchards, farmland, pond surroundings, stream-banks, and roadsides (Main *et al.* 2006). Allelopathic effects are hypothesized to explain the successful invasion of *C. canadensis* (Djurdjević *et al.* 2011; Hu & Zhang 2013). The seedlings of *L. sativa*, one of the most common crops in the region invaded by *C. canadensis*, are sensitive to allelochemicals and the species is widely used as a bioindicator in this type of allelopathy study (Dai *et al.* 2016; Wang *et al.* 2016). The results of the present study can provide a platform for better understanding of the mechanisms of successful invasion of *C. canadensis* and provide an important theoretical foundation and practical significance for effective invasion prevention and control.

This study tested the following hypotheses: First, the allelopathic effects of *C. canadensis* leaf extracts on seed germination and growth of *L. sativa* will increase with increasing latitudinal gradient. This pattern may arise because the leaves of plants that grow in high latitudes secrete higher concentration of allelochemicals compared with those growing in low latitudes due to the lower temperature in high latitudes (Einhellig 1996; Hu & Kong 2002; Erhard & Gross 2005). Second, the allelopathic effects of *C. canadensis* leaf extracts on seed germination and growth of *L. sativa* will increase with increasing invasion degree (i.e., cover of *C. canadensis*), because, for a given land area, the quantity of leaves of the invasive plant species at higher degrees of invasion will be higher than the quantity of leaves at low degrees of invasion, and as a result can release more chemical substances.

## Materials and methods

### Approach

From mid-August 2014 to mid-September 2014, samples of *C. canadensis* (L.) Cronq. were obtained from three

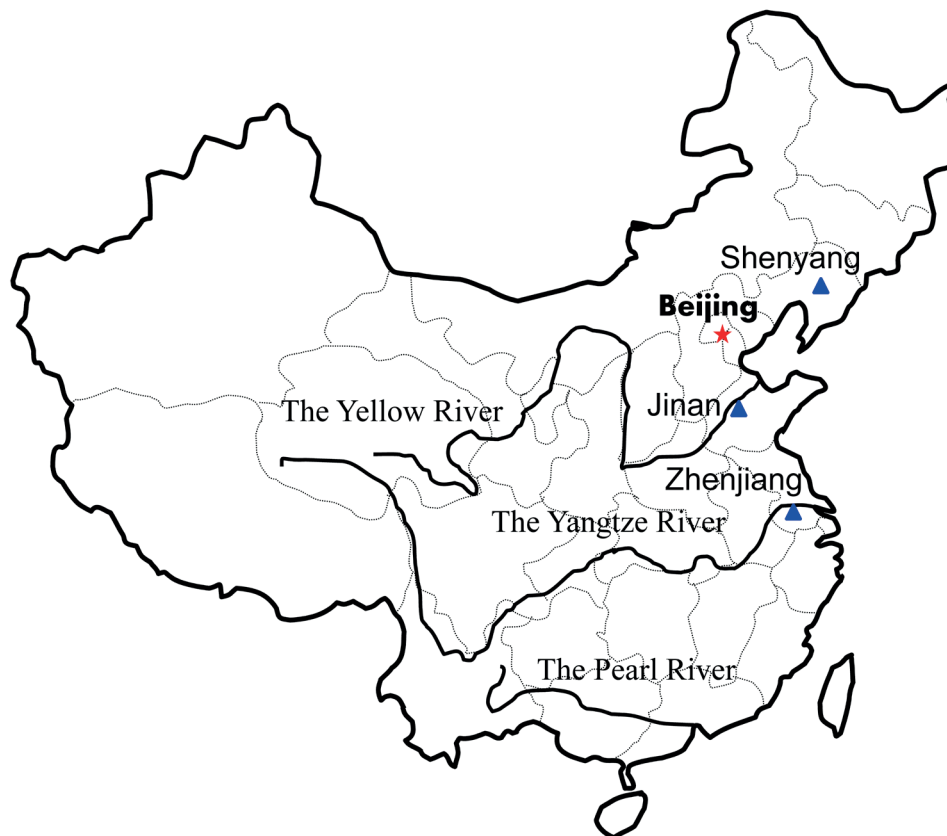
sampling sites along a latitudinal gradient in China. The high latitude site was Shenyang, with a latitude of 41.82°N, 123.46°E and a cold temperate climate. The annual mean temperature of this site is approximately 8.0°C, and the temperature reaches a maximum of 38.0°C and decreases to a minimum of -33.0°C. The annual precipitation at this site is approximately 721.9 mm. The middle latitude site was Jinan, with a latitude of 36.68°N, 116.90°E and a warm temperate climate. The annual mean temperature of this site is approximately 13.8°C, and the temperature reaches a maximum of 42.5°C and decreases to a minimum of -19.0°C. The annual precipitation at this site is approximately 614.0 mm. The low latitude site was Zhenjiang, with a latitude of 32.20°N, 119.51°E and a subtropical monsoon climate. The annual mean temperature of this site is approximately 15.6°C, and the temperature reaches a maximum of 40.2°C and decreases to a minimum of -10.1°C. The annual precipitation at this site is approximately 1088.0 mm. (Site climate summaries are derived from local climatological data.) Figure 1 shows the geographic location of the three sampling sites. Samples were taken from weed communities. The invasion degree of *C. canadensis* was determined to be low (< 35%), moderate (35% to 75%), or high (> 75%) on the basis of its cover classes in each sampling site. Three plant individuals of *C. canadensis* were collected randomly from each of three quadrats (1 m × 1 m) in each of three sampling sites at a given invasion degree (3 plant individuals × 3 quadrat repeats × 3 invasion degrees × 3 sampling sites = 81 plant individuals). The seeds of *Lactuca sativa* L., native to East Asia, were purchased from a local vegetable market. *Lactuca sativa*, one of the most popular vegetables in China, is widely used as both a vegetable and a traditional medicine (Han *et al.* 2010).

After collection, the samples were processed to derive chemical extracts from the leaves. The leaf extract was obtained by placing 10 g of air-dried leaf tissue of *C. canadensis* in flasks containing 500 mL of distilled water and soaked for 48 h at room temperature. The resulting extract was strained through cheesecloth and then through two layers of filter paper to remove solid material. The stock solution was stored at 4°C for no more than one week for further study and diluted with distilled water prior to use. The concentration of leaf extracts was set at 20 g L<sup>-1</sup> with distilled water as control (CK).

### Seed germination experiment

The seed germination treatments included three latitudinal gradient and three invasion degrees. A total of ten treatment combinations were devised as follows: Control (fertilized with sterile distilled water only, CK), SY-L (the extracts of *C. canadensis* leaves collected from Shenyang from locations displaying a low invasion degree), SY-M (the extracts of *C. canadensis* leaves collected from Shenyang from locations displaying a moderate invasion degree), SY-H (the





**Figure 1.** The local geographic location of the three sampling sites. The triangles represent the geographic location of the three sampling sites.

extracts of *C. canadensis* leaves collected from Shenyang from locations displaying a high invasion degree), JN-L (the extracts of *C. canadensis* leaves collected from Jinan from locations displaying a low invasion degree), JN-M (the extracts of *C. canadensis* leaves collected from Jinan from locations displaying a moderate invasion degree), JN-H (the extracts of *C. canadensis* leaves collected from Jinan from locations displaying a high invasion degree), ZJ-L (the extracts of *C. canadensis* leaves collected from Zhenjiang from locations displaying a low invasion degree), ZJ-M (the extracts of *C. canadensis* leaves collected from Zhenjiang from locations displaying a moderate invasion degree), and ZJ-H (the extracts of *C. canadensis* leaves collected from Zhenjiang from locations displaying a high invasion degree). Seed germination experiments were conducted through incubation in Petri dishes (Wang *et al.* 2016). *Lactuca sativa* seeds were surface sterilized (soaked in 1 % NaClO for approximately 15 min) and then washed thoroughly three times with deionized water. Thirty visibly full seeds were placed in a Petri dish of 9 cm diameter and covered with two layers of filter paper. The seeds were treated with 10 mL of *C. canadensis* leaf extracts or sterile deionized water (control, CK). The Petri dishes were placed in a climate-controlled incubator at 27 °C (Thompson *et al.* 1979) for 7 d with 10 h light per day (light intensity was set at 27.5  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ). Five replicate dishes were prepared per treatment.

The number of germinated seeds was counted every day during incubation time, and the seeds were considered germinated when the radicle had emerged from the seed (Wang *et al.* 2016).

### Seedling growth experiment

The same treatments as described above for the seed germination experiment were used for the seedling growth experiment. Ten uniformly sized and strongly growing seedlings per Petri dish were randomly selected for seedling growth measurement at the end of the germination experiment. Seedling height (indicative of the seedling's competitive ability for light; Moles *et al.* 2009), root length (indicative of the seedling's competitive ability for nutrition; James 2008), seedling biomass (fresh weight) (indicative of growth competitiveness; Burns 2004), germination percentage (indicative of the germination ability of seeds), germination potential (indicative of the germination speed and uniformity of seeds), germination index, germination rate index, and vigor index (indicative of the germination speed and vitality of seeds) of *L. sativa* L. were determined. Seedling height and root length were measured using a ruler (Wang *et al.* 2016). Seedling biomass was determined using an electronic balance with an accuracy of 0.001 g (Gong *et al.* 2001; Wang *et al.* 2016).



## Allelopathic suppression by *Conyza canadensis* depends on the interaction between latitude and the degree of the plant's invasion

Germination percentage was calculated using the ratio of the final number of germinated seeds to the total number of the test seeds when no new germination occurred after 7 d of incubation (Wang *et al.* 2016). Germination potential was determined by dividing the number of germinated seeds on the third day by the total number of the test seeds (Wang *et al.* 2016). Germination index was calculated using the following equation: Germination index =  $\sum G_i / I$ , where  $G_i$  represents the number of germinated seeds, and  $I$  represents the number of days after initial seed cultivation (Schmer *et al.* 2012; Hou *et al.* 2014). Germination rate index was calculated using the following equation: GRI = Germination percentage  $\times$  Germination index (Steinmaus *et al.* 2000). Vigor index was determined using the following equation: Vigor index = Seedling biomass  $\times$  Germination index (Lin *et al.* 2000).

### Statistical analyses

Differences among dependent variables were assessed using analysis of variance between treatment groups followed by the Student–Newman–Keuls test for multiple comparisons. Two-way ANOVAs were applied to evaluate the effects of latitudinal gradient and invasion degree on seed germination and growth of *L. sativa* using SPSS Statistics (version 22.0; IBM, Armonk, NY, USA). Statistical significance was set at  $P$  values equal to or lower than 0.05.

## Results

The effects of *C. canadensis* leaf extracts derived from weed populations in the three sampling sites exerted negative effects on root length of *L. sativa* ( $P < 0.05$ ; Tab. 1). The effects of *C. canadensis* leaf extracts from populations in Shenyang exerted negative effects on seedling biomass, germination potential, and germination potential of *L. sativa* ( $P < 0.05$ ; Tab. 1). The effects of *C. canadensis* leaf extracts from populations in Jinan and Shenyang also exerted negative effects on germination percentage, germination index, germination rate index, and vigor index of *L. sativa* ( $P < 0.05$ ; Tab. 1). Meanwhile, the effects of *C. canadensis* leaf extracts from all invasion degrees samples exerted negative effects on root length, germination index, and germination rate index of *L. sativa* ( $P < 0.05$ ; Tab. 2). The effects of *C. canadensis* leaf extracts from low invasion-degree samples also exerted negative effects on vigor index of *L. sativa* ( $P < 0.05$ ; Tab. 2).

The effects of *C. canadensis* leaf extracts on all indices of *L. sativa* increased significantly with increasing latitude across Zhenjiang, Jinan, and Shenyang ( $P < 0.05$ ; Tab. 1). Meanwhile, the effects of *C. canadensis* leaf extracts on root length, germination index, germination rate index and vigor index of *L. sativa* decreased with the different invasion degree ( $P < 0.05$ ; Tab. 2). No significant effect of invasion

degree was observed for seedling height, seedling biomass, germination percentage, and germination potential of *L. sativa* ( $P > 0.05$ ; Tab. 2).

The effects of *C. canadensis* leaf extracts from low and moderate invasion degrees samples in Shenyang on seedling height, root length, seedling biomass, germination potential, germination index, germination rate index, and vigor index of *L. sativa* were significantly higher than those from the same invasion degrees in Zhenjiang and Jinan ( $P < 0.05$ ; Fig. 2). Meanwhile, the effects of *C. canadensis* leaf extracts from low invasion-degree samples in Shenyang on the germination percentage of *L. sativa* were significantly higher than those from the same invasion degree in Zhenjiang and Jinan ( $P < 0.05$ ; Fig. 2). The effects of *C. canadensis* leaf extracts from low invasion-degree samples in the three sampling sites on root length and vigor index of *L. sativa* decreased in the following order: Shenyang  $>$  Jinan  $>$  Zhenjiang ( $P < 0.05$ ; Fig. 2). Meanwhile, the effects of *C. canadensis* leaf extracts from moderate invasion-degree samples in the three sampling sites on seedling height, root length, seedling biomass, germination potential, germination index, germination rate index, and vigor index of *L. sativa* decreased in the following order: Shenyang  $>$  Jinan  $>$  Zhenjiang ( $P < 0.05$ ; Fig. 2). However, there was no significant difference in the effects of *C. canadensis* leaf extracts from high invasion-degree samples on all indices of *L. sativa* among the three sampling sites ( $P < 0.05$ ; Fig. 2).

In Shenyang the effects of *C. canadensis* leaf extracts from low and moderate invasion degrees on seedling height, root length, seedling biomass, germination potential, germination index, germination rate index, and vigor index of *L. sativa* were significantly higher than those from the high invasion degree ( $P < 0.05$ ; Fig. 2). However, there was no significant difference among the three invasion degrees in the effects of *C. canadensis* leaf extracts from populations in Jinan on all indices of *L. sativa* performance ( $P < 0.05$ ; Fig. 2). Meanwhile, for the samples from Zhenjiang, the effects of *C. canadensis* leaf extracts from low and moderate invasion degrees on germination potential and germination index of *L. sativa* were significantly lower than those from the high invasion degree ( $P < 0.05$ ; Fig. 2). In Zhenjiang the effects of *C. canadensis* leaf extracts from low invasion degree on seedling height of *L. sativa* were significantly lower than those from the high invasion degree ( $P < 0.05$ ; Fig. 2). The effects of *C. canadensis* leaf extracts from populations in Zhenjiang on root length of *L. sativa* decreased in the following order: moderate invasion degree  $>$  low invasion degree  $>$  high invasion degree ( $P < 0.05$ ; Fig. 2).

The results of the ANOVAs revealed that latitude and the interaction between latitude and invasion degree exert significant effects on all indices of *L. sativa* growth and germination, while invasion degree significantly affected only seedling height, seedling biomass, and the germination potential of *L. sativa* (Tab. 3,  $P < 0.05$ ).



**Table 1.** Differences in the indices of *L. sativa* under the addition of *C. canadensis* leaf extracts with different latitudinal gradients. Data with different letters in a vertical row indicate a significant difference ( $P < 0.05$ ). Abbreviations: H, seedling height (cm); RL, root length (cm); BM, seedling biomass (g); GPe, germination percentage (%); GPo, germination potential (%); GI, germination index; GRI, germination rate index; VI, vigor index.

	H	RL	BM	GPe	GPo	GI	GRI	VI
Control	2.14±0.11b	3.10±0.49a	0.20±0.01a	90.00±2.79a	76.00±1.63a	52.58±1.40a	47.29±1.77a	10.71±0.58a
Low latitude	3.09±0.07a	1.97±0.08b	0.22±0.01a	86.67±1.22ab	76.67±2.16a	44.49±1.83ab	38.71±1.94ab	9.70±0.55ab
Middle latitude	2.64±0.04a	1.47±0.04c	0.20±0.00a	80.00±1.53bc	74.22±1.47a	39.41±0.77c	31.60±1.06b	7.84±0.28b
High latitude	2.08±0.25b	0.99±0.14d	0.13±0.02b	75.13±4.11c	34.36±8.75b	23.96±4.44d	19.80±4.31c	3.93±1.04c

**Table 2.** Differences in the indices of *L. sativa* under the addition of *C. canadensis* leaf extracts with different invasion degrees. Data with different letters in a vertical row indicate a significant difference ( $P < 0.05$ ). “ns” means no significant difference ( $P > 0.05$ ). Abbreviations: H, seedling height (cm); RL, root length (cm); BM, seedling biomass (g); GPe, germination percentage (%); GPo, germination potential (%); GI, germination index; GRI, germination rate index; VI, vigor index.

	H	RL	BM	GPe	GPo	GI	GRI	VI
Control	2.14±0.11ns	3.10±0.49a	0.20±0.01ns	90.00±2.79ns	76.00±1.63ns	52.58±1.40a	47.29±1.77a	10.71±0.58a
Low degree	2.47±0.24ns	1.35±0.17b	0.17±0.02ns	78.22±3.33ns	55.78±9.03ns	33.49±4.32b	27.86±3.97b	6.65±1.11b
Moderate degree	2.52±0.16ns	1.71±0.15b	0.18±0.01ns	79.49±2.77ns	60.77±7.39ns	36.73±3.92b	30.11±3.79b	7.26±1.06ab
High degree	2.88±0.06ns	1.47±0.06b	0.20±0.01ns	84.67±1.55ns	72.22±2.15ns	39.34±1.47b	33.52±1.77b	8.00±0.44ab

## Discussion

Allelopathy is any beneficial or deleterious effects of plant species on the growth or development of another biological system, through the release of chemicals into the environment. These chemical compounds, known as allelochemicals, are secondary metabolites produced naturally by plant species (Ma *et al.* 2012; Gomaa *et al.* 2014). Previous studies have revealed that the allelopathic effects of invasive plant species, including *C. canadensis* (Djurdjević *et al.* 2011; 2012), on co-occurring species are mainly due to secondary metabolites (Svensson *et al.* 2013; Macel *et al.* 2014).

Consistent with previous reports (Djurdjević *et al.* 2011; Hu & Zhang 2013), our results indicated that *C. canadensis* exerts significant allelopathic effects on seed germination and growth of *L. sativa*. Our findings are consistent with the Novel Weapons Hypothesis, i.e., that some invaders are successful because they possess novel biochemical weapons that function as unusually powerful allelopathic agents, or as mediators of new plant–soil microbial interactions (Callaway & Ridenour 2004).

The invasion of *C. canadensis* currently occurs across a latitudinal gradient in China. The allelopathic effects of *C. canadensis* on the growth of co-occurring species vary across latitudinal gradients due to differences in precipitation, temperature, and sunlight. Latitudinal gradients may affect biomass production and resource allocation (Wright *et al.* 2005; Frenne *et al.* 2013), and these patterns may influence the secretion of allelochemicals (Šežienė *et al.* 2012; Gatti *et al.* 2014). Previous investigations have found that plants that grow in high latitudes pay more allocation of biomass

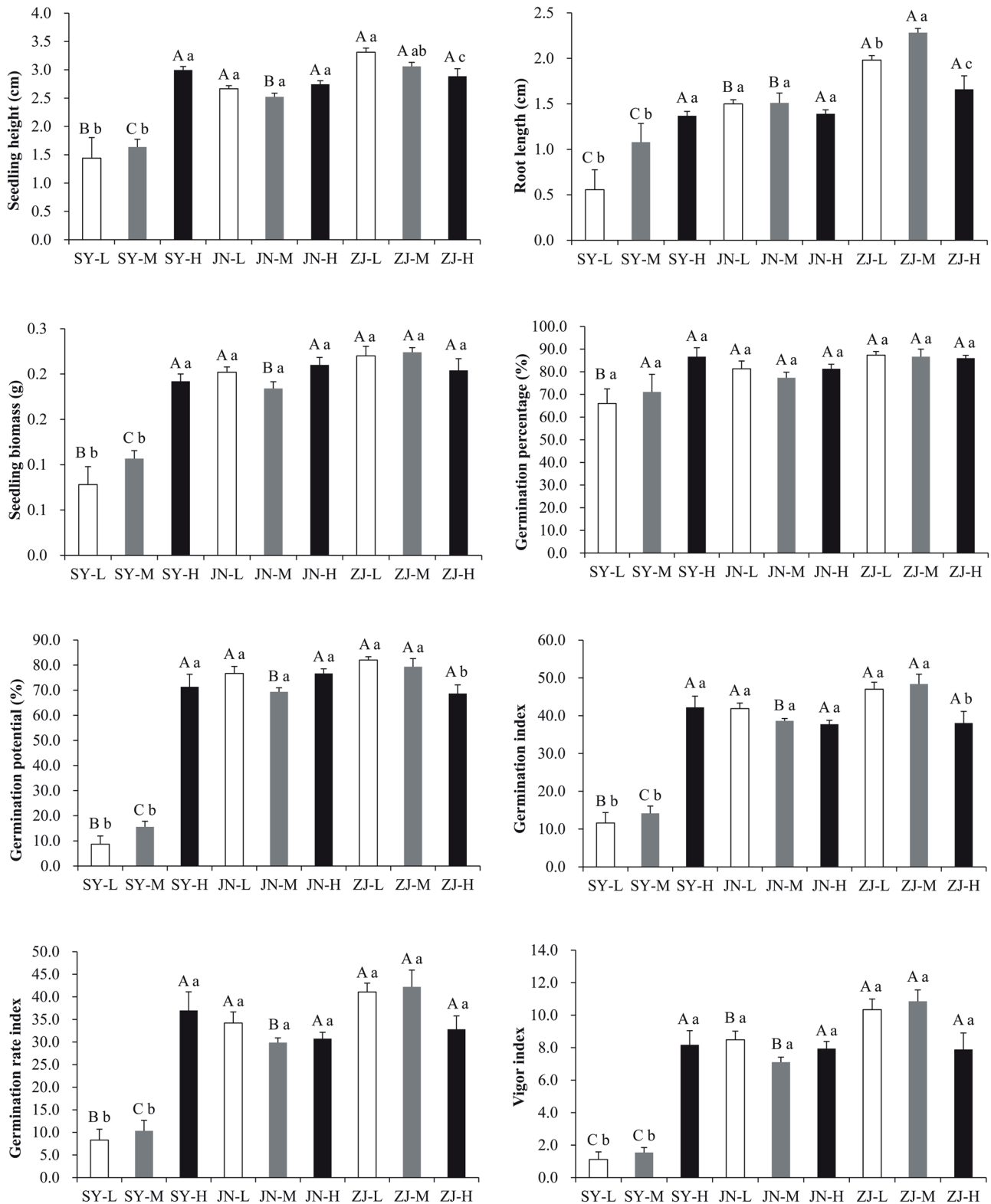
into leaf construction (Wright *et al.* 2005; Reef & Lovelock 2014). Based on this, the leaves of plants that grow in high latitudes are likely to contain higher concentrations of secondary metabolites compared with those growing in low latitudes (Wright *et al.* 2004; Macel *et al.* 2014).

The increase in allelopathic effects of *C. canadensis* leaf extracts on all indices of *L. sativa* with increasing latitudinal gradient (Tab. 1) was consistent with the study’s hypothesis. Previous studies also showed that climatic conditions (such as precipitation and temperature) can affect plant secondary metabolism and change plant allelopathic activity by altering the production of allelochemicals (Šežienė *et al.* 2012; Gatti *et al.* 2014). Meanwhile, as environmental conditions become stressful for plant growth, the allelochemical contents of a plant dramatically increases due to the increase production of carbon-based defensive substances, which are secondary metabolism derivatives (An 2005; Gatti *et al.* 2014). Previous studies also revealed that the concentration of allelochemicals of plant species under low temperature was higher than the concentration under high temperature (Einhellig 1996; Hu & Kong 2002; Erhard & Gross 2005). The phenomenon may also be attributed to the fact that *C. canadensis* is native to Canada (Weaver 2001), which has similar climatic conditions as Shenyang (the high latitude site) due to their similar latitudes. This outcome suggests that the inhibitory allelopathic effects of *C. canadensis* on seed germination and growth of co-occurring species may play an important role in its successful invasion, especially in high latitudes.

Meanwhile, *C. canadensis* displays different invasion degrees in its invaded ecosystems. The allelopathic effects of *C. canadensis* on the growth of co-occurring species may be significantly affected by its invasion degree due to intraspecific competition (Sheppard & Burns 2014).



Allelopathic suppression by *Conyza canadensis* depends on the interaction between latitude and the degree of the plant's invasion



**Figure 2.** The indices of *L. sativa* under the addition of *C. canadensis* leaf extracts with different invasion degrees (L, low; M, moderate; H, high) along a latitudinal gradient (SY, Shenyang; JN, Jinan; ZJ, Zhenjiang). Data with different capital letters indicate a significant difference between the three sampling sites at the same invasion degree and data with different lower case letters indicate a significant difference between the three invasion degrees in the same sampling site ( $P < 0.05$ ). Note: white bar, low invasion degree; grey bar, medium invasion degree; black bar, high invasion degree.

**Table 3.** Two-way ANOVAs on the effects of latitude gradient and invasion stage on the indices of *L. sativa*. Abbreviations: H, seedling height (cm); RL, root length (cm); BM, seedling biomass (g); GPe, germination percentage (%); GPo, germination potential (%); GI, germination index; GRI, germination rate index; VI, vigor index.

		PH	RL	BM	GPe	GPo	GI	GRI	VI
Latitude gradient	<i>F</i>	26.97	38.13	33.04	6.06	96.82	51.46	29.38	44.08
	<i>P</i>	<0.0001	<0.0001	<0.0001	0.0014	<0.0001	<0.0001	<0.0001	<0.0001
Invasion stage	<i>F</i>	4.09	1.52	4.05	1.60	12.84	1.29	1.17	0.89
	<i>P</i>	0.0232	0.2298	0.0240	0.2121	<0.0001	0.2845	0.3196	0.4163
Latitude gradient *	<i>F</i>	12.81	7.28	9.26	2.90	30.34	24.52	15.28	16.15
Invasion stage	<i>P</i>	<0.0001	<0.0001	<0.0001	0.0174	<0.0001	<0.0001	<0.0001	<0.0001

Generally, intraspecific competition increases with increasing invasion degree. As a result, invasive plant species must achieve a higher growth rate to obtain more resources (including space resources and nutrient resources) and achieve as large of a fitness advantage as possible under the changing conditions. As a result, invasive plant species at higher invasion degrees invest low biomass in leaf structures per unit area to gain a higher growth rate compared with plants growing at lower invasion degrees. Previous investigations have also found that the biomass investment per unit of leaf area was correlated positively with population density (Liu *et al.* 2009; Tobin *et al.* 2011). Hence, plants growing at higher invasion degrees may allocate less biomass to leaf construction in order to yield high resource acquisition and use efficiency (James & Drenovsky 2007; Shen *et al.* 2011). Accordingly, the leaves of plants that grow at higher invasion degrees may contain lower concentrations of secondary metabolites compared with plants growing at lower invasion degrees. This is consistent with the finding that the allelopathic effects of *C. canadensis* leaf extracts on seed germination and growth (seedling height, seedling biomass, germination potential, germination index, germination rate index, and vigor index) of *L. sativa* decreased with increasing invasion degree (Tab. 2). The result was inconsistent with the study's hypothesis. It indicates that the inhibitory allelopathic effects of *C. canadensis* on the seed germination and growth of co-occurring species may be attenuated at higher invasion degrees. More importantly, the intensified allelopathic effects of invasive plant species at lower invasion degrees can enable the invader to establish rapidly-growing populations in invaded ecosystems via inhibition of the seed germination and growth of co-occurring species.

### Conclusion

*Conyza canadensis* exhibits negative allelopathic effects on the seed germination and growth of *L. sativa*. This effect increases with increasing latitudinal gradient but decreases with increasing invasion degree. The inhibitory allelopathic effects of *C. canadensis* on the seed germination and the growth of co-occurring species may be crucial to its successful invasion, especially when this species occurs at lower invasion degrees as well as in high latitudes.

## Acknowledgements

This study was supported by National Natural Science Foundation of China (31300343). We are very grateful to the anonymous reviewers for the insightful and constructive comments that greatly improved this manuscript.

## References

- An M. 2005. Mathematical modelling of dose-response relationship (hormesis) in allelopathy and its application. *Nonlinearity in Biology, Toxicology, and Medicine* 3: 153-172.
- Burns JH. 2004. A comparison of invasive and non-invasive dayflowers (Commelinaceae) across experimental nutrient and water gradients. *Diversity and Distributions* 10: 387-397.
- Callaway RM, Ridenour WM. 2004. Novel weapons: invasive success and the evolution of increased competitive ability. *Frontiers in Ecology and the Environment* 2: 436-443.
- Dai ZC, Wang XY, Qi SS, *et al.* 2016. Effects of leaf litter on inter-specific competitive ability of the invasive plant *Wedelia trilobata*. *Ecological Research* 31: 367-374.
- Djurđević L, Gajić G, Kostić O, *et al.* 2012. Seasonal dynamics of allelopathically significant phenolic compounds in globally successful invader *Conyza canadensis* L. plants and associated sandy soil. *Flora* 207: 812-820.
- Djurđević L, Mitrović M, Gajić G, *et al.* 2011. An allelopathic investigation of the domination of the introduced invasive *Conyza canadensis* L.. *Flora* 206: 921-927.
- Einhellig FA. 1996. Interactions involving allelopathy in cropping systems. *Agronomy Journal* 88: 886-893.
- Erhard D, Gross E. 2005. Do environmental factors influence composition of potential allelochemicals in the submersed freshwater macrophyte *Elodea nuttallii* (Hydrocharitaceae)? *Verhandlungen der Internationalen Vereinigung für Limnologie* 29: 287-291.
- Frenne P, Graae BJ, Rodríguez-Sánchez F, *et al.* 2013. Latitudinal gradients as natural laboratories to infer species' responses to temperature. *Journal of Ecology* 101: 784-795.
- Gatti AB, Takao LK, Pereira VC, Ferreira AG, Lima MIS, Gualtieri SCJ. 2014. Seasonality effect on the allelopathy of cerrado species. *Brazilian Journal of Biology* 74: S064-S069.
- Gomaa NH, Hassan MO, Fahmy GM, González L, Hammouda O, Atteya AM. 2014. Allelopathic effects of *Sonchus oleraceus* L. on the germination and seedling growth of crop and weed species. *Acta Botanica Brasílica* 28: 408-416.
- Gong P, Wilke BM, Strozzi E, Fleischmann S. 2001. Evaluation and refinement of a continuous seed germination and early seedling growth test for the use in the ecotoxicological assessment of soils. *Chemosphere* 44: 491-500.
- Han YF, Cao GX, Gao XJ, Xia M. 2010. Isolation and characterisation of the sesquiterpene lactones from *Lactuca sativa* L. var. *anagustata*. *Food chemistry* 120: 1083-1088.





## Allelopathic suppression by *Conyza canadensis* depends on the interaction between latitude and the degree of the plant's invasion

- Hao JH, Qiang S, Chrobock T, Kleunen M, Liu QQ. 2011. A test of Baker's law: breeding systems of invasive species of Asteraceae in China. *Biological Invasions* 13: 571-580.
- Hou QQ, Chen BM, Peng SL, Chen LY. 2014. Effects of extreme temperature on seedling establishment of nonnative invasive plants. *Biological Invasions* 16: 2049-2061.
- Hu F, Kong C. 2002. Allelopathy of *Ageratum conyzoides*. VI. Effects of meteorological conditions on allelopathy of *Ageratum conyzoides*. *The Journal of Applied Ecology* 13: 76-80.
- Hu G, Zhang ZH. 2013. Aqueous tissue extracts of *Conyza canadensis* inhibit the germination and shoot growth of three native herbs with no autotoxic effects. *Planta Daninha* 31: 805-811.
- James JJ. 2008. Leaf nitrogen productivity as a mechanism driving the success of invasive annual grasses under low and high nitrogen supply. *Journal of Arid Environments* 72: 1775-1784.
- James JJ, Drenovsky RE. 2007. A basis for relative growth rate differences between native and invasive forb seedlings. *Rangeland Ecology & Management* 60: 395-400.
- Lin WX, Kim KU, Smin DH. 2000. Rice allelopathic potential and its modes of action on barnyardgrass (*Echinochloa crusgalli*). *Allelopathy Journal* 7: 215-224.
- Liu TF, Zhang CL, Yang GS, et al. 2009. Central composite design-based analysis of specific leaf area and related agronomic factors in cultivars of rapeseed (*Brassica napus* L.). *Field Crops Research* 111: 92-96.
- Ma JH, Xing GE, Yang WX, et al. 2012. Inhibitory effects of leachate from *Eupatorium adenophorum* on germination and growth of *Amaranthus retroflexus* and *Chenopodium glaucum*. *Acta Ecologica Sinica* 32: 50-56.
- Macel M, Vos RCH, Jansen JJ, Putten WH, Dam NM. 2014. Novel chemistry of invasive plants: exotic species have more unique metabolomic profiles than native congeners. *Ecology and Evolution* 4: 2777-2786.
- Main CL, Steckel LE, Hayes RM, Mueller TC. 2006. Biotic and abiotic factors influence horseweed emergence. *Weed Science* 54: 1101-1105.
- Moles AT, Warton DI, Warman L, et al. 2009. Global patterns in plant height. *Journal of Ecology* 97: 923-932.
- Reef R, Lovelock CE. 2014. Historical analysis of mangrove leaf traits throughout the 19th and 20th centuries reveals differential responses to increases in atmospheric CO<sub>2</sub>. *Global Ecology Biogeography* 23: 1209-1214.
- Schmer MR, Xue Q, Hendrickson JR. 2012. Salinity effects on perennial, warm-season (C4) grass germination adapted to the northern Great Plains. *Canadian Journal of Plant Science* 92: 873-881.
- Seastedt TR, Pyšek P. 2011. Mechanisms of plant invasions of North America and European grasslands. *Annual Review of Ecology Evolution and Systematics* 42: 133-153.
- Šežienė V, Baležentienė L, Ozolinčius R. 2012. Allelopathic impact of some dominants in clean cuttings of Scots pine forest under climate change conditions. *Ekologija* 58: 59-64.
- Shah MA, Callaway RM, Shah T, et al. 2014. *Conyza canadensis* suppresses plant diversity in its nonnative ranges but not at home: a transcontinental comparison. *New Phytologist* 202: 1286-1296.
- Shen XY, Peng SL, Chen BM, et al. 2011. Do higher resource capture ability and utilization efficiency facilitate the successful invasion of native plants?. *Biological Invasions* 13: 869-881.
- Sheppard CS, Burns BR. 2014. Effects of interspecific alien versus intraspecific native competition on growth of native woody plants. *Plant Ecology* 215: 1527-1538.
- Steinmaus SJ, Timonhy SP, Jodie SH. 2000. Estimation of base temperature for nine weed species. *Journal of Experimental Botany* 51: 275-286.
- Svensson JR, Nylund GM, Cervin G, Toth GB, Pavia H. 2013. Novel chemical weapon of an exotic macroalga inhibits recruitment of native competitors in the invaded range. *Journal of Ecology* 101: 140-148.
- Thompson PA, Cox SA, Sanderson RH. 1979. Characterization of the germination responses to temperature of lettuce (*Lactuca sativa* L.) achenes. *Annals of Botany* 43: 319-334.
- Tobin PC, Berec L, Liebhold AM. 2011. Exploiting Allee effects for managing biological invasions. *Ecology Letters* 14: 615-624.
- Turk MA, Tawaha AM. 2003. Allelopathic effect of black mustard (*Brassica nigra* L.) on germination and growth of wild oat (*Avena fatua* L.). *Crop Protection* 22: 673-677.
- Wang CY, Xiao HG, Zhao LL, et al. 2016. The allelopathic effects of invasive plant *Solidago canadensis* on seed germination and growth of *Lactuca sativa* enhanced by different types of acid deposition. *Ecotoxicology* 25: 555-562.
- Wang CY, Zhou JW, Liu J, Wang L, Xiao HG. 2017. Reproductive allocation strategy of two herbaceous invasive plants across different cover classes. *Polish Journal of Environmental Studies* 26: 355-364.
- Weaver SE. 2001. The biology of Canadian weeds. 115. *Conyza canadensis*. *Canadian Journal of Plant Science* 81: 867-875.
- Weber E, Sun SQ, Li B. 2008. Invasive alien plants in China: diversity and ecological insights. *Biological Invasions* 10: 1411-1429.
- Weiner J, Wright DB, Castro S. 1997. Symmetry of below-ground competition between *Kochia scoparia* individuals. *Oikos* 79: 85-91.
- Wright IJ, Reich PB, Cornelissen JHC, et al. 2005. Modulation of leaf economic traits and trait relationships by climate. *Global Ecology Biogeography* 14: 411-421.
- Wright IJ, Reich PB, Westoby M, et al. 2004. The worldwide leaf economics spectrum. *Nature* 428: 821-827.
- Xie Y, Li ZY, Gregg WP, Dianmo L. 2001. Invasive species in China-an overview. *Biodiversity and Conservation* 10: 1317-1341.
- Yan XL, Liu QR, Shou HY, et al. 2014. The categorization and analysis on the geographic distribution patterns of Chinese alien invasive plants. *Biodiversity Science* 22: 667-676.
- Zaplata MK, Winter S, Biemelt D, Fischer A. 2011. Immediate shift towards source dynamics: the pioneer species *Conyza canadensis* in an initial ecosystem. *Flora* 206: 928-934.

