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Allelopathic effects of extracts from *Solidago canadensis* L. against seed germination and seedling growth of some plants

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Abstract: To investigate the potential role of allelopathy in plant interference and in the successful invasion of alien species *Solidago canadensis*, aqueous and ethanolic extracts from rhizomes, stems and leaves of *S. canadensis* were prepared and used as treatment solutions to assess their effects on seed germination and seedling growth in four target species, mulberry (*Morus alba*), morning glory (*Pharbitis nil*), wheat (*Triticum aestivum*) and rape (*Brassica campestris*). Reduction and/or growth in germination and growth of the target plant species in the presence of both aqueous and ethanolic extracts at different concentrations indicated that the responses were species-specific and concentration-dependent. Generally, ethanolic extracts (especially from leaves) imposed stronger effects on both seed germination and seedling growth. Extracts with lower concentration at 0.001 g/ml dw could stimulate the seedling growth of rape and morning glory, whereas extracts at any given concentrations have inhibitory effects on wheat and mulberry. It is suggested that the aqueous and ethanolic extracts of all the three parts of *S. canadensis* have significant allelopathic effects. Although both inhibition and stimulation occurred in the germination and growth of the target species, extracts with higher concentrations definitely inhibit seed germination and seedling growth of all target plants. We suggest that allelopathy plays a more important role than other mechanisms do in the out-competition of *S. canadensis* over other plants, and make it invasive in new habitats.
Keywords: Canada goldenrod(*Solidago canadensis* L.); allelopathy; extracts; seed germination; seedling growth

Introduction

Plant invasion is considered the second most serious threat to natural habitats, after habitat fragmentation and loss (Randall, 1996), which causes severe ecological damage and worldwide economic loss. Currently, invasive alien plants are regarded as a major threat to natural areas due to their abilities to change the community structure within their new habitats and to displace native vegetation (Xie *et al.*, 2000).

Canadian goldenrod(*Solidago canadensis* L.) is a perennial herbaceous plant of family Compositae, and is considered native to North America. This species was introduced into China as an ornamental plant in the earlier 1930s and now it becomes naturalized in Shanghai, Jiangsu, Zhejiang and other regions after its introduction, invasion, establishment and propagation in 1980s (Guo and Fang, 2003; Jin *et al.*, 2004). Therefore, *S. canadensis* is regarded as an invasive alien plant species in China(Richardson *et al.*, 2000).

Nowadays, *S. canadensis* has appeared in different habitats from abandoned farmlands, roadsides to nurseries, tea gardens, orchards, and even sparse forests, and evolves into a pernicious weed in its invasive habitats. More importantly, it can jeopardize landscaping and planting by invading into green fences or green belts in city. In the past 2—3 years, it has fallen into the most problematic invasive alien species in these regions, and its quick dispersal and great damage to the ecological integrity have developed into environmental issues. Although the impacts of invasive *S. canadensis* are felt from the local to national scale, and have raised public attention, *S. canadensis* is still spreading radially near the bigger cities. Our field survey on the occurrence of *S. canadensis* carried out earlier this year showed that in its invasive areas, particularly on abandoned farmlands, this species was markedly abundant and out-competed other plants. Accordingly, overall species abundance and diversity were reduced significantly. This observation is consistent with the common notions that rapid growth and reproduction allow invasive plants to overwhelm and displace native species, and to threaten the integrity of natural ecosystem (Callaway, 2002). Therefore, invasive success of alien *S. canadensis* and its destruction to native biological diversity have been considered to be a serious environmental problem concerning plant invasion in China.

To cope with this troublesome problem, the most immediate question to be answered is the causes of *S. canadensis*'s successful invasion. So far, the mechanisms that allow *S. canadensis* to dominate native communities have not been well understood. Allelopathy is an important mechanism of plant interference mediated by the addition of plant-produced phytotoxins to the plant environments (Alam *et al.*, 2001), which was thought to be the primary reason that some alien plant species can invade new habitats and out-compete native species (Callaway and Aschehoug, 2000). Compared with its closely related species such as *S. altissima* and *S. gigantean*, *S. canadensis* is a less studied species in terms of plant invasions. No matter what systematic or taxonomic relations they have, they are very close to

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each other. Considering the allelopathic effect of *S. altissima* on rice and ragweed(Ito *et al.*, 1998) and the close relation between *S. altissima* and *S. canadensis*, it appears appropriate for us to speculate that *S. canadensis*'s successful invasion as alien species may be attributed to its intrinsic allelopathy. Thus, the objective of this study was to experimentally verify this speculation. To do so, we selected mulberry (*Morus alba*); morning glory (*Pharbitis nil*), wheat (*Triticum aestivum*) and rape (*Brassica campestris*) as target plants to investigate the potential allelopathic effects of *S. canadensis* on seed germination and seedling growth, and to explore the underlying cause that this alien plant has become such a successful invasive species in China.

1 Materials and methods

1.1 Sampling and processing of vegetative parts from *S. canadensis*

Vegetative parts of *S. canadensis* plants(20—40 cm in height), which were reproduced from creeping rhizomes after the first year of growth, were sampled on abandoned farmlands. The collected vegetative parts were sorted into rhizomes, stems and leaves. After dried for 72 h in oven at 40℃, these parts were processed into powder by milling (2400 r/min for 4 min) in a herbal medicine grinder (Type FM177, Tianjin Taisite Instrument Co., Ltd). The powder thus instantly passed through the 100-mesh sieve, and kept for use.

1.2 Seeds of tested target plants

The seeds of rape, wheat were purchased from a seed company, whereas the seeds of morning glory and mulberry were the kind gift from Dr. Lu X P, Zhejiang University.

1.3 Preparation of extracts from three vegetative parts

Aqueous and ethanolic extracts were prepared respectively from the rhizomes, stems and leaves of *S. canadensis* by the procedures of water-decocting and ethanol circumfluence described as follows: (1) Water-decocting method: weigh out precisely 10 g of powder of rhizomes, stems and leaves, transfer respectively to a clean 250 ml Erlenmeyer flask containing 150 ml distilled water, place the flask into an 100℃ water bath for 8 h to decoct, filter through Watman No. 1 filter paper, condense the filtrate to a 100-ml volume by rotary evaporator to make 0.1 g/ml dw stock aqueous extracts. (2) Ethanol circumfluence method: weigh out precisely 10 g of powder of rhizomes, stems and leaves, transfer respectively to soxhlet distillation device with 250 ml ethanol and distill for 24 h. After distillation, evaporate completely by rotary evaporator. Prepare 0.1 g/ml dw ethanolic extracts as stock solution by dissolving in 100 ml distilled water.

1.4 Trail for seed germination

From stock solutions, both the aqueous extracts and ethanolic extracts were diluted with autoclaved distilled water to achieve a total of three concentrations at 0.1, 0.01, 0.001 g/ml dw respectively. To make seed bed for germination, two pieces of filter paper with an absorbent cotton pad underlay were put on the base of 9 cm petri dish, and well moistened with 5 ml diluted extracts (the same volume of distilled water as control (CK)). 50 sterile seeds of each species(except the case of morning glory where 30 seeds were used due to bigger in size) were spread evenly on seed bed inside the Petri dish under sterile conditions, and then exposed to 12 h light and 12 h darkness in a growth chamber at 25℃ and more than 75% relative humidity for germination. Three replicates were performed for each treatment. 72 h or more after incubation, the germination was evaluated in terms of number and length of radicle. For each species, seeds were considered germinated when the radicle was longer than seed. The inhibitory rate (expressed as inhibitory percentage) of seed germination was calculated according to the following equation as described by Lin *et al.*(2000). IR (%) = $(T_i\text{-}T_0)/T_0 \times 100$, where IR is the inhibitory rate, T_i denotes the value of germination rate given by treatment, and T_0 is the value given by control.

1.5 Trail for seedling growth

The objective of this trial was to determine the growth responses of each target plant species to increasing concentrations of aqueous and ethanolic extracts from *S. canadensis*. To achieve this, sand culture experiments were carried out in a growth chamber. Before sowing, the seeds were incubated at room temperature for 2—3 d to make the radicle protrude the seed coat. Half-strength Hoagland nutrient solution, prepared according to the modified recipe (Olsen and Sommers, 1982), was used to make the treatment solutions of aqueous and ethanolic extracts to concentrations at 0.1, 0.01, 0.001 g/ml dw, respectively. Quartz sand was washed sequentially with tap and deionized water until free from amorphous matter and other impurities, and autoclaved sufficiently. 1.5 kg dry quartz sand was weighed into polystyrene vessels with enough holes to allow free drainage of treatment solutions, and wet with 500 ml of the respective treatment solutions of aqueous and ethanolic extracts. The seeds with sprouted radicle (just visible expression of radicle) were sowed directly in the sand, and the vessels were placed in growth chambers at 25℃ with alternant 12 h light and 12 h darknes. For each treatment, 30 seeds were applied and 3 replicates were given. 4—5 d later, the length of radicle and that of hypocotyl (or coleoptile, in the case of wheat) were separately measured.

1.6 Data analysis

Data are mean values for all the experiments and were analyzed by a simple one-way analysis of variance. For the differences among different treatments, multiple comparisons were performed by SSR method.

2 Results

2.1 Effects of *S. canadensis* extracts on seed germination of target plants

The effects of different extracts from three parts of *S. canadensis* at different concentrations on seed germination of target plants are shown in Table 1. The results show that the minimum effective concentration for inhibitory impact on wheat seed germination was between 0.1 and 0.01 g/ml dw, and that on mulberry was higher than 0.01 g/ml dw for ethanolic extracts or a little higher than 0.01 g/ml dw for aqueous extracts ($p<0.05$). However, this was not the case when the effective concentrations on rape and morning glory were evaluated, extracts with concentration lower than 0.01 g/ml dw have significant positive stimulation on seed germination ($p<0.05$), however, reduction of germination still occurred when higher concentration was given. In conclusion, *S. canadensis* extracts exhibited species-specific and concentration-dependent effects.

Table 1 Effects of different extracts from three parts of *S. canadensis* at different concentrations on seed germination of target plants

Extraction methods	Organs extracted	Extract concentration, g/ml dw	Germination rates of target plants, %			
			Mulberry	Morning glory	Wheat	Rape
Aqueous extracts	Rhizome	0.1	0.0 ± 0.0^{e}	0.0 ± 0.0^{d}	50.0 ± 3.3^{d}	56.0 ± 1.6^{c}
		0.01	60.4 ± 1.6^{b}	49.4 ± 4.1^{b}	85.0 ± 3.3^{b}	94.7 ± 0.9^{a}
		0.001	73.4 ± 3.3^{a}	53.8 ± 1.6^{a}	97.3 ± 2.5^{a}	99.3 ± 0.9^{a}
	Stem	0.1	0.0 ± 0.0^{e}	0.0 ± 0.0^{d}	40.0 ± 1.6^{d}	20.7 ± 0.9^{d}
		0.01	58.7 ± 0.9^{c}	49.3 ± 1.9^{b}	86.0 ± 1.6^{b}	96.0 ± 1.8^{a}
		0.001	75.3 ± 0.9^{a}	54.0 ± 1.6^{a}	95.3 ± 0.9^{a}	96.0 ± 2.6^{a}
	Leaf	0.1	0.0 ± 0.0^{e}	0.0 ± 0.0^{d}	6.0 ± 0.9^{e}	18.0 ± 1.6^{d}
		0.01	44.7 ± 0.9^{d}	50.7 ± 0.6^{b}	70.0 ± 2.5^{c}	74.7 ± 1.9^{b}
		0.001	68.8 ± 1.6^{ab}	59.3 ± 0.9^{a}	93.4 ± 2.5^{a}	86.0 ± 4.3^{a}
Ethanolic extracts	Rhizome	0.1	0.0 ± 0.0^{e}	0.0 ± 0.0^{d}	16.7 ± 2.9^{e}	16.3 ± 0.5^{d}
		0.01	60.0 ± 3.3^{b}	46.9 ± 1.6^{b}	76.7 ± 1.9^{c}	70.7 ± 2.9^{b}
		0.001	70.0 ± 3.3^{ab}	48.0 ± 1.6^{b}	97.3 ± 2.5^{a}	96.0 ± 1.6^{a}
	Stem	0.1	0.0 ± 0.0^{e}	0.0 ± 0.0^{d}	18.0 ± 1.6^{e}	0.0 ± 0.0^{d}
		0.01	65.3 ± 0.9^{b}	46.7 ± 2.5^{b}	76.7 ± 2.5^{c}	76.0 ± 1.6^{b}
		0.001	72.9 ± 0.9^{a}	53.4 ± 1.9^{ab}	98.0 ± 1.6^{a}	96.0 ± 0.8^{a}
	Leaf	0.1	0.0 ± 0.0^{e}	0.0 ± 0.0^{d}	0.0 ± 0.0^{e}	0.0 ± 0.0^{d}
		0.01	30.7 ± 0.9^{d}	32.7 ± 1.9^{c}	66.0 ± 1.6^{cd}	50.0 ± 3.3^{c}
		0.001	69.3 ± 2.5^{ab}	59.3 ± 2.5^{a}	94.0 ± 2.5^{a}	79.3 ± 2.5^{b}
Control			74.7 ± 0.9^{a}	50.7 ± 1.6^{b}	98.0 ± 1.6^{a}	78.7 ± 0.9^{b}

Notes: Average values for germination rates (%) of three replicates, along with standard errors, are presented; the uppercase and/or lowercase letters behind each value denote statistically very significant differences($p<0.01$) and significant differences($p<0.05$), respectively. The values with the same letter within the same column are not significantly different

Also, as shown in Table 1, for each target plant, all extracts from different organs at the same concentration have parallel effects. Taken wheat as an example, no matter what organs to be extracted, when 0.1 g/ml dw extract was applied, seed germination was greatly inhibited, whereas when extracts from all three organs at concentration lower than 0.01 g/ml dw were adopted, there were no obvious differences in inhibitory effects among them, except that 0.01 g/ml dw ethanolic extract from leaf resulted in significant reduction of seed germination($p<0.05$). In spite of this, it could be notably found that both aqueous and ethanolic leaf extracts exerted stronger influence over seed germination than rhizome and stem extracts did. Similar results could be found in other target plants.

Moreover, when the two methods for extracting were synthetically compared, it could be found that ethanolic extracts showed somewhat stronger inhibitory effect than aqueous extracts do. Particularly in the case of wheat, all ethanolic extracts from different organs exhibited stronger significantly than

those aqueous ones at each given concentration ($p<0.05$).

Fig.1 shows the collective pattern of inhibitory effects based on the inhibitory rate. As far as inhibitory percentage of seed germination is concerned, it was clear that all extracts with concentration above 0.01 g/ml dw resulted in significant inhibition on all target plants; when the concentration was elevated to 0.1 g/ml dw, all extracts (both aqueous and ethanolic extracts from all organs) gave rise to complete inhibitory effects on seed germination of mulberry and morning glory. Considering differences between extracts from different organs as a whole, it could be found that ethanolic leaf extract presented the strongest inhibition, which gave 100% inhibition on seed germination of all target plants. Furthermore, there existed label bars in Fig.1 that were below or above the horizontal line when treated with 0.001 g/ml dw extracts, which implied that seed germination was inhibited(in the case of bar above) or stimulated(in the case of bar below). It was suggested that treatment with extract at 0.001 g/ml dw might produce inhibitory or stimulated effects on seed germination of plants. Among the four target plants, stimulation of seed germination occurred in rape and morning glory, while inhibition arose in wheat and mulberry at the same concentration of 0.001 g/ml dw.

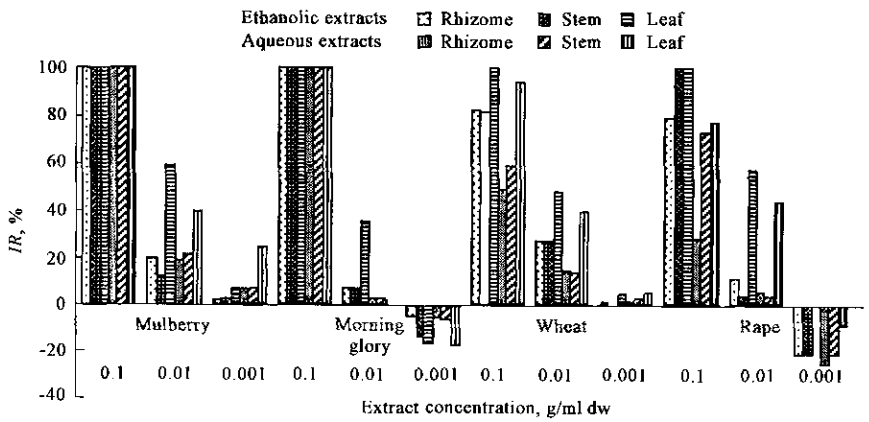

Fig.1 Inhibitory rates (*IR*) of seed germination of target plants by treatment with *S. canadensis* extracts at different concentrations

2.2 Effects of *S. canadensis* extracts on seedling growth of target plants

The length of radicle and that of hypocotyl (or coleoptile, in the case of wheat) are two important parameters for seedling growth. Data of radicle length and hypocotyl length were calculated statistically shown in Fig.2. Compared with the control, which was treated with Hoagland nutrient solution free of extracts, it was found that the radicle growth of wheat, rape and mulberry was inhibited more or less by all extracts at any given concentrations, while that of morning glory was inhibited significantly only by extracts at concentrations higher than 0.01 g/ml dw ($p<0.05$), which indicated that different plants exhibited different sensitivities to treatment of *S. canadensis* extracts.

As far as the growth of hypocotyl was concerned, a phenomenon in point was the stimulation in morning glory and rape by lower concentration extracts of 0.001 g/ml dw ($p<0.05$). In addition, it is obvious that coleoptile growth of wheat was greatly inhibited by all extracts at each concentration. As for the case of mulberry, its hypocotyl growth reduced when all extracts, except for those 0.001 g/ml dw aqueous extracts, were applied. Moreover, it appeared that ethanolic extracts imposed stronger effects on both radicle and hypocotyl growth than aqueous ones did.

3 Discussion

Allelopathic effects of invasive species and native species have been extensively studied since the definition was adopted (Alam *et al.*, 2001). The successful spreading of *S. canadensis* can be explained by their small achene with long pappus and by vegetative growth after establishment. However, it is not easy to explain the mono-dominant patches in its invasive habitats. The evolution of increased competitive ability (EICA) hypothesis has been proposed and widely used to explain the successful invasion of several alien species (Shea and Chesson, 2002). However, the mechanism behind invasive success of *S. canadensis* is very complex, and EICA hypothesis probably can not give a satisfactory explanation. van Kleunen and Schmid (2003) found that no direct evidence for an evolutionary increased competitive ability in the invasive *S. canadensis*, and they thought other mechanisms rather than EICA may have played a more critical role in the successful worldwide invasion of *S. canadensis*.

Our present results revealed that both seed germination and seedling growth of the selected target plants could be inhibited by extracts of *S. canadensis*,

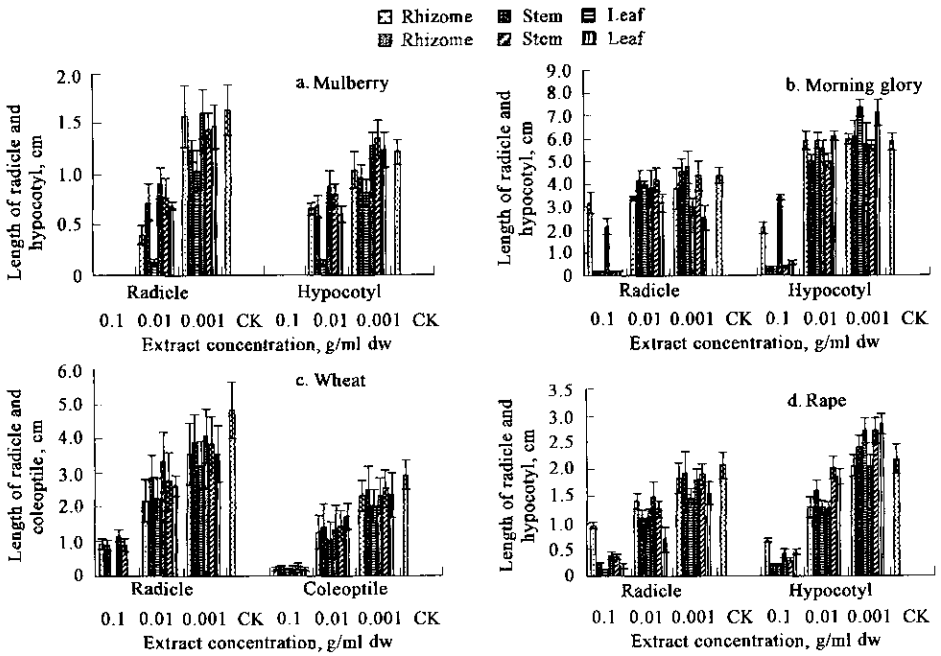

Fig.2 Effects of different extracts of *S. canadensis* at different concentrations on radicle and hypocotyl(or coleoptile) growth of target plants
The legend is the same as Fig.1

especially by those from leaves, which verified that *S. canadensis* created efficient allelopathic effects on other competitors. The allelopathic efficiency is at least 5 times high of *Wedelia chinensis* (Zeng *et al.*, 1996) and similar to tomato (Zhou *et al.*, 1997). Our field survey also showed that only a few plant species, such as *Geranii caroliniani* and *Alopecurus aequalis*, co-occurred with *S. canadensis* and poorly grew. These results strongly implied that allelopathy played a critical role in the course of successful invasion of *S. canadensis*.

Interestingly, our results also indicated that 0.001 g/ml dw extracts could significantly produce inhibitory or stimulated effects on target plants. Similar effects were reported by Bhatia *et al.*(1982), and they found stimulating effect of *Chenopodium album* on the growth of wheat. The mechanism of this phenomenon and its significance in the plant-plant interaction remain to be discovered.

It is generally accepted that allelochemicals were exudated by roots or leached from leaves and targeted to restrict the growth of other plants (Rice, 1984). In our study, another result deserved to be emphasized is that leaf extract from *S. canadensis* exhibited stronger allelopathic activity than rhizome and stem extracts, the mechanism of which is not fully elucidated and should be disclosed by isolating the allelochemicals and understanding their metabolism and biosynthesis. Only by accomplishing this can we disclose the mode of allelopathic action, comprehend the underlying causes of invasion of *S. canadensis*, and thus focus on its control and restoration of native habitats.

So far, several exotic plant species naturalized in China have been proved to possess allelopathic actions on other organisms including other plants, animals and bacteria. *Ambrosia artemisiifolia* and *A. trifida*, which are two foreign weeds of North American origin, were confirmed to restrain their surrounding plants by allelopathic effects through root exudation or leaf volatilization (Wang, 1995). *Ageratum conyzoides* is native to Mexico, and is an invasive weed as well in China (Kong *et al.*, 1999). Recently, *Eupatorium adenophorum* and *Mikania micrantha*, which are believed to be invasive alien plants of Middle American origin, were also found to be allelopathic to native species in their new habitats (Song *et al.*, 2000; Zhang *et al.*, 2002). Our present study proved that *S. canadensis*, which is also an invasive plant of North American origin, imposed allelopathic effects on its potential competitors. All these reports strongly implied that allelopathy plays an important role in exotic plant invasion (Wang *et al.*, 2004).

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