



Allelopathy of the invasive plant *Bidens frondosa* on the seed germination of *Geum japonicum* var. *chinense*

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ABSTRACT. Five gradient concentrations (0.02, 0.04, 0.06, 0.08, and 0.10 g/mL) of leaching liquors from the roots, stems, and leaves of the invasive plant *Bidens frondosa* were used as conditioning fluid to examine its influence on seed germination conditions of the native plant *Geum japonicum* var. *chinense* in Huangshan. All leaching liquors of organs suppressed the seed germination of *Geum japonicum* var. *chinense* and reduced the final germination percentage and rate, and increased the germination inhibition rate, with a bimodal dependence on concentration. The leaching liquor inhibited the seed germination significantly at the concentration of 0.02 g/mL respectively. The seed germination was also inhibited as the concentration reached to 0.04 g/mL and beyond. Hence the allelopathic effects of the organs were significantly enhanced respectively. This phenomenon represented the presence of allelopathy substances in the root, stem and leaf of *Bidens frondosa*.

Key words: *Bidens frondosa*, allelopathy; Invasive plants; *Geum japonicum* var. *chinense*; Seed germination

INTRODUCTION

Allelopathy was first proposed by the Austrian scientist Molisch in 1937 (Jiang et al., 2006) and refers to the phenomenon that plants release chemicals into the surroundings, affecting the growth and development of themselves and of other plants (Yu et al., 1991). This is an ecological mechanism for natural chemical regulation and the adaptation of plants in an ecosystem (Lv and Duan, 2005), which affects chemical ecology defense and competition. Allelopathy is an important means of adaptation, survival, and competition, and exists widely in nature; it has become a domestic and international concern, and various studies have been conducted to address these concerns (Jiang et al., 2010). Currently, a number of alien invasive weeds has been confirmed to show allelopathy, such as *Solidago Canadensis*, and *Mikania*, among others (Zeng and Luo, 1993; Wang and Zhu, 1996; Hu and Kong, 1997; Shao et al., 2003; Su and Zhu, 2003; Mei et al., 2005; Wan et al., 2011; Lu et al., 2011).

Bidens frondosa is the Asteraceae *Bidens* annual herb that grows to 30-150 cm and shows a high reproductive ability. A normal developing plant can produce hundreds of thousands of seeds, which are large, light, and have barbs. They fall from the plant easily upon reaching maturity and can be dispersed through wind and currents, as well as by adhering to animals and humans.

The bryophyte *B. frondosa* is also highly adaptable and is widely distributed in nature. It is capable of growing in wet or shallow water conditions in temperate or subtropical climates, as commonly observed in Huangshan, China. It also may invade Huangshan scenic locations. *Geum japonicum* var. *chinense* is native to Huangshan and is a perennial herb that grows in or near hillside meadows, fields, rivers, thickets, and thin forest (Flora Editorial Board of Chinese Academy of Sciences, 2004).

Based on a 4-year investigation from 2009-2013 of Huangshan Scenic locations, a considerable amount of *B. frondosa* was found to have invaded the natural distribution area of *G. japonicum* var. *chinense*, indicating sympatric distribution. In this study, *G. japonicum* var. *chinense* seeds were used as the receptor and the leaching liquor of the roots, stems, and leaves of *B. frondosa* were used as the conditioning fluid. We examined the allelopathy of *B. frondosa* and developed technical support for using the allelochemicals for the production of biological herbicides with new target points or for the synthetic template of new chemical herbicides. It is important to reduce the dependence of agricultural ecosystems on chemical pesticides and provide a reference for preventing the alien weed from invading scenic locations in Huangshan.

MATERIAL AND METHODS

Plant materials

The seeds of *B. frondosa* and *G. japonicum* var. *chinense* were respectively sampled from the city of Huangshan and the Beihai Scenic Area in September and October of 2011.

Optimum temperature and light conditions for seed germination

Through a single factor experiment of seed germination of *G. japonicum* var. *chinense*, the optimum temperature and light conditions were determined for seed germination,

which were similar to the environmental conditions in the allelopathic experiment.

Water temperature setting

Seeds of *G. japonicum* var. *chinense* were screened and disinfected using hydrogen peroxide. The seeds were then sterilized in 5 culture dishes and covered with a layer of filter paper and absorbent cotton. Fifty seeds were chosen randomly, placed in each dish, and placed in an illumination incubator at temperatures of 20, 22, 24, 26, and 28°C while keeping other environmental conditions consistent. The germination was checked once a day and recorded. This experiment was repeated 3 times.

Light setting

Based on the above experiment, a culture under dark conditions was used as a control. The 5 temperatures were considered to be the experimental groups.

Preparation of leaching liquor from *B. frondosa*

B. frondosa were rinsed using the distilled water. The roots, stems, and leaves were cut into 3-5 mm pieces. Next, 20 g each sample was mixed with 200 mL sterile distilled water to prepare the liquor. Samples were shaken for 10 min every 12 h. After 48 h, 4 layers of sterile gauze were used to filter the aqueous extracts and remove plant residues. Samples were centrifuged for 15 min at 3000 rpm to obtain the mother liquor of leaching liquor with 0.10 g/mL concentration (Wang et al., 2012). The mother liquor was diluted to 100, 80, 60, 40, and 20% with sterile distilled water and the 3 organ samples were treated with the 5 concentration of mother liquors. The control group was treated with sterile distilled water and placed in freezer in (1°C).

Determination of germination of *Geum japonicum* var. *chinense* seeds

The Petri dish and filter paper were sterilized. The seeds were also disinfected for 10 min with 10% H₂O₂ and then washed 3 times with distilled water (3 min each wash). Fifty *G. japonicum* var. *chinense* seeds were selected and placed in the filter paper of the Petri dish. Next, 5 mL leach liquor at the 5 different concentrations was added to each of the 3 organ samples, while 5 mL sterile distilled water was added to the control sample. This procedure was repeated 3 times. The Petri dishes were wrapped to prevent water evaporation and contamination, and then randomly placed into illumination incubator at 26°C (determined through the single-factor test). Seed germination was monitored every 24 h and terminated when there were no newly germinated seeds for 5 days. Germination was considered to occur when the radicle broke the seed coat by 1-2 mm.

Data analysis

The treatment group and control group were analyzed based on a previously described equation (Du et al., 2009).

$$GR = G/50 \quad (\text{Equation 1})$$

$$RG = \sum_i 1(N_i/D_i) \quad (\text{Equation 2})$$

$$PI(-) = UG(AE)/UG(CK)-1 < 0 \quad (\text{Equation 3})$$

$$PS(+) = UG(AE)/UG(CK)-1 > 0 \quad (\text{Equation 4})$$

In these formulas, *GR* represents germination rate, *G* represents the number of germinated seeds, *UG* represents the ultimate germination rate, *RG* represents seed germination rate, *D_i* represents the number of days, *N_i* represents the number of germinated seeds on the *i*th day, *PI* represents the inhibition ratio of seed germination, *PS* represents the promotion rate of seed germination, *UG* (AE) represents the ultimate germination rate after treatment with leaching liquor, *UG* (CK) represents the ultimate germination rate in the control group, *PI*, and *PS* is the conversion into percentages.

RESULTS

Influence of different leaching liquors on germination of *G. japonicum* var. *chinense* seeds

Based on the test results, the influence of the leaching liquor with different concentrations of 3 organs from *B. frondosa* on the germination of *G. japonicum* var. *chinense* seeds is shown in Table 1.

Table 1. Influence of leaching liquor containing different concentrations of roots, stems, and leaves of *Bidens frondosa* on the germination of *Geum japonicum* var. *chinense* seeds.

Treatment	Germination					
	Distilled water	0.02 g/mL	0.04 g/mL	0.06 g/mL	0.08 g/mL	0.10 g/mL
Root	98.00	98.00 ± 1.63	46.00 ± 2.83	58.00 ± 1.63	46.00 ± 2.83	24.00 ± 4.90
Stem	98.00	76.00 ± 0.82	56.00 ± 2.45	74.00 ± 2.83	50.00 ± 1.63	24.00 ± 3.27
Leaf	98.00	95.00 ± 2.45	54.00 ± 4.89	68.00 ± 1.63	44.00 ± 2.83	26.00 ± 3.27

Table 1 indicates that leaching liquor containing different concentrations of various organs significantly inhibited seed germination. Generally, germination rate decreased with increasing leaching liquor concentration. When the concentration was 0.10 g/mL, germination rate reached its lowest rate; the difference in the leaching liquors from roots, stems, and leaves at different concentrations was highly significant ($P < 0.05$); when the concentration was 0.02 g/mL, the stem leaching liquor inhibited germination more significantly than using liquors of roots and leaves; however, when the concentration was 0.04 g/mL, seed germination was inhibited more strongly than when the concentration was 0.06 g/mL. This requires further study.

Influence of different concentrations of leaching liquor on the germination rate of *G. japonicum* var. *chinense* seeds

The germination rate of allelopathy was calculated according to the test results, as shown in Table 2.

Table 2 shows that the germination rate of the control group (distilled water) was 48.64 seeds/day. When the concentration of root leaching liquor was 0.02 g/mL, the difference in seed germination rate between the experimental and control groups was not very significant ($P > 0.05$). The leaching liquors of other organs of different concentrations showed significant or extremely significant differences in germination rate. With increasing leaching liquor concentration, germination was more strongly inhibited; the maximum effect was observed at a leaching liquor concentration of 0.10 g/mL.

Table 2. Influence of leaching liquor containing different concentrations of roots, stems, and leaves of *Bidens frondosa* on the germination rate of *Geum japonicum* var. *chinense* seeds.

Treatment	Rate of germination (seeds/day)					
	Distilled water	0.02 g/mL	0.04 g/mL	0.06 g/mL	0.08 g/mL	0.10 g/mL
Root	48.64 ± 0.54	47.75 ± 0.21	13.92 ± 0.07	18.83 ± 0.10	17.10 ± 0.33	7.44 ± 0.05
Stem	48.64 ± 0.54	16.36 ± 0.52	14.67 ± 0.17	21.82 ± 0.68	11.72 ± 0.27	5.32 ± 0.24
Leaf	48.64 ± 0.53	35.74 ± 0.42	17.70 ± 0.25	21.04 ± 0.44	13.26 ± 0.20	6.58 ± 0.07

Inhibition/promotion of leaching liquor on *G. japonicum* var. *chinense* seeds

The ratio of germination to the promotion ratio of seeds was determined (Table 3).

As shown in Table 3, in addition to the root extract concentration of 0.02 g/mL, leaching liquor of other concentrations inhibited seed germination; with increasing concentration, the absolute value of germination rate also increased. When the leaching liquor of roots, stems, and leaves was the highest concentration tested of 0.10 g/mL, seed germination inhibition rates were -75.51, -75.51, and -73.47%, respectively. Generally, leaching liquor inhibited seed germination, but the inhibition was bimodal; when the concentration was 0.04 g/mL and 0.10 g/mL, the inhibition rate of seed germination had a larger absolute value.

Table 3. Influence of roots, stems, and leaves of *Bidens frondosa* on the germination rate/promotion rate of *Geum japonicum* var. *chinense* seeds.

Treatment	Percentage inhibition (-) or promotion (+)					
	Distilled water	0.02 g/mL	0.04 g/mL	0.06 g/mL	0.08 g/mL	0.10 g/mL
Root	0	0	-53.06	-40.82	-53.06	-75.51
Stem	0	-22.45	-42.86	-24.49	-48.98	-75.51
Leaf	0	-3.06	-44.90	-30.61	-55.10	-73.47

DISCUSSION

Our results showed that the *B. frondosa* significantly inhibited the germination of *G. japonicum* var. *chinense* when the concentration of the stem leaching liquor was greater than 0.02 g/mL and those of the root and leaf leaching liquors were greater than 0.04 g/mL. The inhibition reached a maximum at a concentration of 0.10 g/mL. All organs of *B. frondosa*

inhibited the germination of *G. japonicum* var. *chinense* seeds at different concentrations of leaching liquor, reducing the seed germination rate. These results support the allelopathic effects on plant growth, which is consistent with the phenomenon observed in the natural environment. Additionally, the strength of allelopathy differed among plant organs and with varying concentrations of leaching liquor; generally, the effect was stronger with increased leaching liquor concentration. *B. frondosa* can release water-soluble substances to create an environment conducive to their growth by rain and other effects to enhance their survival, explaining the invasion mechanism of *B. frondosa* (Du et al., 2009).

In terms of inhibition of seed germination of *G. japonicum* var. *chinense*, the leaching liquors from the organs of *B. frondosa* not only reduced the final germination rate and increased the inhibition rate of seed germination, but also reduced the seed germination rate. The allelopathy of plants is widespread in nature and plays an important role in the formation of dominant species, community succession, and revegetation. Plants interact with each other by releasing secondary metabolism compounds to protect their favorable ecological environment (Gu et al., 1998). Terpenoids are abundant in higher plants, mainly in the form of volatile oil, which can protect against damage from insects. These substances can produce allelopathic effects in other plants after their release into the environment (Hu and Kong, 2002). The toxicity of plant terpenoid compounds is selective, which is manifested as the inhibition of plant germination and growth at high concentrations. Furthermore, the inhibition is gradually strengthened with increasing concentrations of allelochemicals. This can promote growth at low concentrations, which has been observed in allelopathy research of *odorata*, *cairica*, and *Wedelia* (Zeng et al., 1996; Liu et al., 1997; He et al., 2002; Fang et al., 2005). Further in-depth analysis of the chemical composition and release mechanism of allelopathy of *B. frondosa* is needed.

The leaching liquors of *B. frondosa* organs contain water-soluble allelopathic substances. With respect to the various organs of *B. frondosa*, the leaching liquors of the root, stem, and leaf inhibited seed germination to varying degrees at the same concentration, indicating there are some differences between the allelopathy of different plant parts, which may be due to differences in their allelochemical levels. The allelopathy strength of the leaching liquors of various organs is generally stronger with increasing leaching liquor concentrations, which is relatively strong at a concentration of 0.04 g/mL.

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