Plant Ecology and Evolution 144 (3): 275–280, 2011
doi:10.5091/plecevo.2011.459

PLANT ECOLOGY EVOLUTION

REGULAR PAPER

Phytotoxic effects of a dominant weed *Ligularia virgaurea* on seed germination of *Bromus inermis* in an alpine meadow community

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Background and aims – *Ligularia virgaurea* is an invasive weed species in the alpine meadow community that contains allelopathic compounds, which may affect seed germination of other co-existent species.
Material and methods – In a laboratory study, allelopathic effects of root, stem and leaf aqueous extracts of *Ligularia virgaurea*, were evaluated on seed germination of *Bromus inermis*, a native grass species.
Key results – Results showed that root, stem and leaf extracts of *Ligularia virgaurea* significantly decreased the germination percentage, relative germination percentage, germination index and weighted germination index of *Bromus inermis*. First germination time and mean germination time of *Bromus inermis* were also delayed. The concentration of the extracts had no significant effects on the germination indices. The type of extract (root, stem or leaf), on the contrary, showed the following trend on the mean relative germination percentage: RGP leaf extract treatment > RGP stem extract treatment > RGP root extract treatment. The response index values of root, stem and leaf aqueous extracts differ as follows: RI root extracts > RI stem extracts > RI leaf extracts.
Conclusion – Our results demonstrate that aqueous extracts of *Ligularia virgaurea* have a significant allelopathic inhibition on seed germination of *Bromus inermis*. The root extracts of *Ligularia virgaurea* have a greater inhibition effect on seed germination of *Bromus inermis*, relative to stem and the leaf extracts.

Key words – alpine meadow, *Bromus inermis*, germination, invasive weed, *Ligularia virgaurea*, phytotoxic effects.

INTRODUCTION

In plant-plant interactions, plants show effects (inhibition or enhancement) on the growth of neighboring plants through the release of chemical compounds, called allelochemicals. This phenomenon is known as allelopathy (Rice 1984). Allelopathy plays an important role, at least in part, in the biological invasion and success of exotic plant species in the new environment to out-compete native species (Hierro & Callaway 2003). Allelopathic effects are attributed to the presence of allelochemicals, or mixtures of allelochemicals, that have additive or synergistic activity (Lin et al. 2004, Tesio et al. 2008). Higher plants may release allelochemicals by volatilization and leaching from leaf and plant litter, root exudation, and decomposition and leaching from soil-incorporated tissues (e.g. Birkett et al. 2001, Fujii et al. 2004). Many studies on allelopathy dealt with cultivated farmland systems and focused on the interaction between crops and weeds (e.g. Einhellig 1996, Tesio et al. 2008). In the last two decades, allelopathic potential has been demonstrated in numerous weed and crop species in natural as well as agro-ecosystems (Batish et al. 2001). Subsequently, allelopathy experiments were carried out on grassland and forest ecosystems. Allelopathic effects between species within alpine grassland communities has been reported in several studies (e.g. Zhang et al. 1989, Bai & Zhang 1994, Ma et al. 2005, Tang et al. 2008) and few have reported the allelopathic potential of invasive weed species. Noxious and invasive weeds exhibit allelopathic potential through the release of allelochemicals from roots, stems, leaves, rhizomes, flowers, fruits or seeds, which effect upon seed germination and seedling survival of neighboring species (e.g. Ahn & Chung 2000, Batish et al. 2007, Duke et al. 2007).

Ligularia virgaurea Mattf. ex Rehder & Kobusi is a typical invasive species in alpine meadow grassland, which belongs to the Asteraceae family propagating by its rhizomes as well as by sexual reproduction (Wu et al. 2010a). This plant is present in Bhutan, Nepal and China where it is recorded from western Gansu, Qinghai, northwestern Sichuan, northwestern Yunnan and Tibet. It is found at marsh edges, foothill meadows and ditches from 2,600 to 4,700 m elevation. *Ligularia virgaurea* is a typical and dominant noxious weed in the alpine meadow community of the Qinghai-Tibetan Plateau and had strong invasion abilities (Wu et al. 2010a). Ma et al. (2005) reported that the major allelopathic substances present in *Ligularia virgaurea* include phenolic, syringic, ferulic and chlorogenic acids. On the other hand, the typical local breeding grass of *Bromus inermis* Leyss. (Poaceae) is the main forage grass widely distributed in the alpine meadow community of the Qinghai-Tibetan Plateau. This perennial grass species is an important component of the alpine meadow community and is an important food source for animals. It has been observed that *Bromus inermis* populations decline in the alpine meadow community when the *Ligularia virgaurea* population increases (Wu et al. 2009), which might indicate that *Bromus inermis* does not tolerate the allelopathic substances produced by *Ligularia virgaurea*.

Different tissues may have different contents of allelopathic substances and present different effects depending on the species (Khanh et al. 2005). For *Ligularia virgaurea*, there is little information on the allelopathic effects of the different tissues on *Bromus inermis*. Therefore, the present study was conducted to: (1) evaluate the allelopathic effects of aqueous extracts of *Ligularia virgaurea* on *Bromus inermis*; (2) compare the allelopathic activity of aqueous extracts of *Ligularia virgaurea* obtained from root, stem and leaf. The study will contribute to understand the mechanism behind the decline of *Bromus inermis* populations within the alpine meadow community after the invasion of *Ligularia virgaurea*.

MATERIAL AND METHODS

Plant species and study site

Individuals of *Ligularia virgaurea* were collected from six different populations in the alpine meadow community in August 2009. The fresh individuals were taken to the laboratory for experiments. The receptor seeds of *Bromus inermis* were collected from the same alpine meadow community in the same study area in October 2009. Mature seeds of *Bromus inermis* were brown and were collected randomly from more than thirty individuals for each species to get an adequate representation of the whole community. Seeds of *Bromus inermis* were cleaned and spread on tables at room temperature (approximately 15°C) until they were used.

The site was located at mean 3500 m a.s.l. at Maqu (33°42'21"N 102°07'02"E) in Gansu Province, P.R. China, which is located at the eastern Qinghai–Tibetan Plateau. The mean annual temperature is 1.2°C, ranging from - 10°C in January to 11.7°C in July, with about 270 frost days. The mean annual precipitation is 620 mm, with the main rain period during a short and cool summer. The annual cloud-free solar radiation was about 2580 h. The vegetation is typical alpine meadow and is dominated by clonal grasses (*Kobresia* sp., grasses and sedges) and forbs (*Saussurea* sp. and other species of Asteraceae).

Aqueous extraction

The plant tissues of *Ligularia virgaurea* were washed thoroughly with tap water and rinsed with distilled water. The

Table 1 – Mean value (± SE) of germination percent (GP, %), relative germination percent (RGP, %), first germination time (FGT, d), mean germination time (MGT, d), germination index (GI) and weighted germination index (WGI) for seeds of *Bromus inermis* under different extracting solution concentrations for root, shoot and leaf of *Ligularia virgaurea*.

Treatments		GP	RGP	FGT	MGT	GI	WGI
Control	0 g/L	19.22 ± 3.26	-	5.17 ± 0.41	8.55 ± 1.51	1.19 ± 0.13	0.16 ± 0.03
Root	10g/L	6.11 ± 2.10	31.67 ± 4.50	22.67 ± 4.79	26.13 ± 2.27	0.24 ± 0.11	0.04 ± 0.03
	20g/L	2.75 ± 1.15	14.00 ± 2.08	23.33 ± 3.32	23.83 ± 3.82	0.07 ± 0.05	0.02 ± 0.00
	40g/L	1.37 ± 1.19	7.33 ± 1.35	9.67 ± 2.66	9.67 ± 2.66	0.08 ± 0.01	0.01 ± 0.00
	80g/L	0.79 ± 0.47	4.00 ± 1.93	13.00 ± 2.52	13.00 ± 2.51	0.01 ± 0.00	0.003 ± 0.00
Shoot	10g/L	8.80 ± 4.75	45.67 ± 4.54	4.67 ± 1.15	8.98 ± 2.29	0.71 ± 0.14	0.07 ± 0.02
	20g/L	7.58 ± 3.44	51.67 ± 5.87	5.67 ± 2.08	9.52 ± 0.50	0.37 ± 0.16	0.07 ± 0.02
	40g/L	9.03 ± 3.24	47.00 ± 4.22	8.67 ± 0.58	15.32 ± 1.17	0.31 ± 0.15	0.06 ± 0.02
	80g/L	4.01 ± 3.45	21.00 ± 3.19	12.00 ± 3.81	14.33 ± 6.50	0.18 ± 0.09	0.03 ± 0.01
Leaf	10g/L	15.78 ± 4.26	59.67 ± 5.78	12.67 ± 2.07	19.60 ± 2.32	0.56 ± 0.05	0.09 ± 0.03
	20g/L	13.62 ± 3.71	71.00 ± 4.92	4.33 ± 1.15	9.53 ± 2.92	0.71 ± 0.03	0.12 ± 0.03
	40g/L	8.25 ± 2.27	43.00 ± 5.53	7.33 ± 0.58	14.00 ± 2.30	0.37 ± 0.07	0.07 ± 0.02
	80g/L	13.39 ± 0.34	69.50 ± 5.50	8.00 ± 0.00	10.98 ± 1.31	0.66 ± 0.01	0.11 ± 0.00

Table 2 – Results of ANOVA tests for the effects of plant tissues and extracting solution gradient on germination percent (GP, %), relative germination percent (RGP, %), first germination time (FGT, d), mean germination time (MGT, d), germination index (GI) and weighted germination index (WGI) for *Bromus inermis* in this study. All values show significance at P ≤ 0.05.

	GP		RGP		FGT		MGT		GI		WGI	
	F	*P*-value	*F*	*P*-value	*F*	*P*-value	*F*	*P*-value	*F*	*P*-value	*F*	*P*-value
Plant tissues (PT)	9.772	**0.001**	8.910	**0.001**	3.085	0.061	1.027	0.371	7.300	**0.003**	10.488	**0.000**
Concentration (C)	2.035	0.131	1.841	0.167	0.309	0.819	0.485	0.695	2.900	0.052	2.220	0.107
PT*C	1.183	0.342	1.259	0.313	0.792	0.583	0.958	0.470	1.069	0.404	1.367	0.261

plant tissues were clipped into root, stem and leaf sections. Root, stem and leaf of *Ligularia virgaurea* were used in this study. Their aqueous extracts were obtained by soaking 24 g fresh leaves in 96 ml distilled water (dilution 20% w/w) in flasks, shaken for 7 h on orbital shaker at + 4°C, to avoid fermentation of raw material (Tesio et al. 2008). The aqueous extracts were diluted appropriately with sterile distilled water and contained four concentration regimes of 10, 20, 40 and 80 g/L for root, stem and leaf and a control treatment with sterile distilled water (0 g/L).

Germination experiment

Five ml extracts were applied to Petri dish (9.0 cm diameter) lined with double layer of filter paper. Petri dishes with sterile distilled water were used as control. Seeds of *Bromus inermis* were scarified for 40 min in concentrated sulfuric acid before the experiment, to remove seed dormancy and enhance water adsorption (Tesio et al. 2008). Fifty seeds of each species were used in each Petri dish, and three replicates of each treatment including control sample. The seed germination experiment were incubated in Growth Chamber and temperature was controlled by a thermo period of 25/5°C (12 h light/12 h dark) in order to simulate the condition of natural alpine area. Petri dishes were moistened by different concentration of water extract separately. Every day, the number of germinated seeds was recorded. Newly emerged seedlings were removed from the Petri dishes, and seeds were regularly watered with distilled water. A seed was considered germinated when the radicle was visible.

Statistical analysis

In this study, we used following germination parameters: Germination percentage (GP, %), Relative germination percentage (RGP), First germination time (FGT), Mean germination time (MGT), Germination index (GI), Weighted germination index (WGI), and Response index (RI). Final percentage germination (GP) for each treatment was calculated after sixty days. The germination index (GI) is based on number of seeds that germinated and the germination rate. These parameters were also calculated from the formulas proposed by Figueroa & Armesto (2001), Bu et al. (2007a, 2007b, 2008, 2009), and Wu & Du (2008):

GP = 100 × GN / SN; GN is the total number of germinated seed, SN is the total number of seeds tested.

RGP = GP under aqueous extraction treatment / GP under sterile distilled water treatment (CK) × 100.

FGT = Number of days from seed sowing to first germination.

$$\mathrm{MGT} = \sum_i G_i \times i \Big/ \sum_i G_i$$

where *i* is the number of days since the day of sowing (day 0) and G_i is the number of seeds germinated on day *i*. Only seeds that germinated were included in the calculation.

$$\mathrm{GI} = \Big(\sum_i (60 - i) \times G_i\Big) \times 100 \Big/ (60 \times \mathrm{GN})$$

GI is a synthetic measure designed to reflect the synthetical germination ability including germination rate and germination numbers. Where *i* is the number of days since the day of sowing and G*i* is the number of seeds germinated on day *i*.

A weighted germination index (WGI) as described by Bu et al. (2009) was calculated with maximum weight given to the seeds germinating early and less to those germinating late.

$$\mathrm{WGI} = [60 \times n_1 + 59 \times n_2 + 58 \times n_3 + \ldots\ldots + 1 \times n_{60}] / (60 \times N)$$

where n_1, n_2, …, n_{60} are the number of seeds that germinated on first, second, and subsequent days until the 60^{th} day, respectively; 60, 59, ……, 1 are the weights given to the seeds germinated on first, second, and subsequent days until the 60^{th} day. N is the total number of seeds placed in incubation. Response index (RI): RI = 1 - (C/T) (If T > C) and RI = (T/C) - 1 (If T < C); RI ranges from - 1 to + 1, with positive values indicating stimulation by the treatments and negative values indicating inhibition by them, relative to the controls. The absolute value of RI means the degree of inhibition and stimulation of aqueous extracts (Tang et al. 2008).

Significant differences for all statistical tests were evaluated at the level of P ≤ 0.05 with ANOVA. All data analyses were conducted using SPSS for Windows, Version 13.0 (Chicago, IL, USA).

RESULTS

Effects of *Ligularia virgaurea* extracts on germination indices of *Bromus inermis*

Results showed that root, stem and leaf extracts of *Ligularia virgaurea* decreased GP, RGP, GI and WGI and delayed the FGT and MGT of *Bromus inermis* (table 1). Mean RGP val-

Table 3 – Inhibition index value (RI) of aqueous extracts of *Ligularia virgaurea* on seed germination percentage (GP), first germination time (FGT), mean germination time (MGT), germination index (GI) and weighted germination index (WGI) of *Bromus inermis*.

Treatments		RI-GP	RI-FGT	RI-MGT	RI-GI	RI-WGI
Root	10g/L	- 0.68	0.77	0.67	- 0.80	- 0.75
	20g/L	- 0.86	0.78	0.64	- 0.94	- 0.88
	40g/L	- 0.93	0.47	0.12	- 0.93	- 0.94
	80g/L	- 0.96	0.60	0.34	- 0.99	- 0.98
Shoot	10g/L	- 0.54	- 0.10	0.05	- 0.40	- 0.56
	20g/L	- 0.61	0.09	0.10	- 0.69	- 0.56
	40g/L	- 0.53	0.40	0.44	- 0.74	- 0.63
	80g/L	- 0.79	0.57	0.40	- 0.85	- 0.81
Leaf	10g/L	- 0.18	0.59	0.56	- 0.53	- 0.44
	20g/L	- 0.29	- 0.16	0.10	- 0.40	- 0.25
	40g/L	- 0.57	0.29	0.39	- 0.69	- 0.56
	80g/L	- 0.30	0.35	0.22	- 0.45	- 0.31

ues showed the following trend: leaf extract treatment > stem extract treatment > root extract treatment. GP, GI and WGI also revealed important variation in function of the extraction type (table 1). Significant differences between the control treatment (CK) and the extracts of root, shoot and leaf of *Ligularia virgaurea* in GP, RGP, GI and WGI were shown. Different plant tissues had significant effects on GP, RGP, GI and WGI, but the concentration gradient had no significant effects on the germination indices used in this study (table 2).

Effects of *Ligularia virgaurea* extracts on the inhibition index value (RI) of *Bromus inermis*

RI values showed that aqueous extracts of root, shoot and leaf of *Ligularia virgaurea* all present obvious inhibition effects on GP, GI and WGI, and stimulation effects on FGT and MGT of *Bromus inermis*. The RI values differ in function of the extract type (root, leaf or shoot) as follows: root extracts > stem extracts > leaf extracts (table 3). Different concentrations had no significant influence on the germination parameters here considered.

DISCUSSION

Ligularia virgaurea is a frequent and dominant noxious weed in the alpine meadow community of the Qinghai-Tibetan Plateau (Ma et al. 2005, Wang et al. 2008, Wu et al. 2010a). But the allelopathic behavior of *Ligularia virgaurea* has been rarely investigated. Our results showed that the aqueous extracts of different tissues of *Ligularia virgaurea* all presented a significant inhibition effect on the seed germination characteristics of *Bromus inermis*. However, the different concentrations of aqueous extracts had no significant effects on its seed germination. Based on our results, it is demonstrated that aqueous extracts of *Ligularia virgaurea* have important negative effects on seed germination indices of *Bromus inermis* and this inhibition is much stronger with root extracts than with extracts of above ground tissues.

Muller (1966) had suggested that allelopathic effects among species may be a potential mechanism for transformation of vegetation composition. Tesio et al. (2008) had reported that laboratory bioassays were used as functional tools for detecting preliminarily allelopathic potential in higher plant species. In this study, the inhibitions were significant for the germination percentage and germination index, but not significant for germination times. Differences in sensitivity among various germination indices may exist (Chiapusio et al. 1997). The decrease of GP and the delay of FGT and MGT revealed that the allelopathic behavior of *Ligularia virgaurea* had significant inhibition effects for germination rate and germination time of *Bromus inermis*. Values of GI and WGI are synthetic measures designed to reflect the germination ability with the inclusion of germination rate and germination time (Bu et al. 2009, Wu & Du 2008). Variation of GI and WGI among treatments suggests that the allelopathic effects of *Ligularia virgaurea* have a negative impact on the germination dynamics of *Bromus inermis*. This is in agreement with field observations that show that only a few *Bromus inermis* individuals persist in meadow communities infested by *Ligularia virgaurea*. Inhibitory effects obtained in laboratory studies do not provide immediate evidence that allelopathy is actually operational in field settings. However, laboratory bioassays are fast and repeatable tools for investigating the potential role of allelochemical interactions (Inderjit & Weston 2000, Tesio et al. 2008). Reduction of *Bromus inermis* individuals around *Ligularia virgaurea* population was also related to grazing disturbance because *Ligularia virgaurea* populations significantly take advantage of lowered competition with *Bromus inermis* when the latter is grazed (Wu et al. 2009).

The calculated inhibition indices in our results revealed that the root extracts of *Ligularia virgaurea* have greater inhibition effects on seed germination of *Bromus inermis*, relative to the extracts of stems and leaves of *Ligularia virgaurea*. This indicates that allelopathic interactions among species mainly come from root, but not above ground litter.

Allelopathic effects of *Ligularia virgaurea* can play a role in the germination dynamics of *Bromus inermis* in alpine meadow communities. Infestations of *Ligularia virgaurea* are basically structured in black-soil patches, as it is often noted in many perennial weed infestations (Wu et al. 2010a, 2010b). The bioactivity of plant tissues may increase its fitness for a patch structure, operating in addition to the reproduction advantage with both sexual and asexual recruitment (Wu et al. 2010a). Eventually, all these results proved substantial modifications in the equilibrium of mature plant communities (Zimdahl 1999, Fujii et al. 2004, Tesio et al. 2008).

Ligularia virgaurea allelopathic extracts, particularly from roots, significantly inhibited the germination of *Bromus inermis*. And, further studies are indeed necessary to elucidate the potential of *Ligularia virgaurea* allelopathy in field conditions, including the identification of the major chemical compounds responsible for the phenomenon and the explanation of coexistence mechanism in the field of *Ligularia* *virgaurea*-invaded community based on the interaction of allelopathy.

ACKNOWLEDGEMENTS

The authors thank D.A. Sampietro, Steven Dessein, Muhammad N. Mushtaq and two anonymous reviewers for their valuable comments on the manuscript and thank Mr. Liu ZH for the assistance with field seed collection and Wang MR, Gao T with the lab experiment. The study was supported by Projects of Natural Science Foundation of China (NSFC30900177), and funds of the State Key Laboratory of Soil Erosion and Dryland Farming on the Loess Plateau (10502-T1) and NWSUAF (Z111020903).

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Manuscript received 7 Jun. 2010; accepted in revised version 29 Apr. 2011.

Communicating Editor: Steven Dessein.