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Seed-to-seed potential allelopathic effects between *Ligularia virgaurea* and native grass species of Tibetan alpine grasslands

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Abstract Allelopathy is an important process in plant communities, but the role of seed allelopathy in natural ecosystems remains poorly understood. In the present study, we examined the potential allelopathic effects of *Ligularia virgaurea* (a dominant species in degraded Tibetan grasslands) seeds on the germination of four native grass species (*Festuca sinensis*, *Agrostis gigantea*, *Bromus inermis*, and *Elymus nutans*). The results showed that *L. virgaurea* seeds can have potential allelopathic effects on seed germination, mean time to germination and root growth rates of native grass species. We further demonstrate that these effects are driven by a water-soluble seed leachate. Species with smaller seeds were generally more sensitive than larger seeded species. The results suggest that seed-to-seed allelopathic potential may be an important mechanism driving the dominance of *L. virgaurea* in degraded alpine grasslands on the Tibetan Plateau. Further studies are required to demonstrate effects of seed-to-seed potential allelopathy in a field setting.

Keywords Allelopathy · Seed leachate · Seed germination · Root growth · *Ligularia virgaurea* · Alpine meadow

Introduction

Allelopathy is defined as the influence of one plant on another through the release of chemical compounds

under natural conditions (Rice 1984). The main sources of allelopathic compounds are live and dead plant tissues, including roots (Javaid et al. 2006; Mutlu and Atici 2009), stems (Farooq et al. 2008; Han et al. 2008), rhizomes (Yoko et al. 2001; Vrchatová and Šerá 2008), leaves (Macías et al. 1993; Chaves and Escudero 1997; Jefferson and Pennacchio 2003), and flowers and inflorescences (Ballester et al. 1982; Wakjira et al. 2005). Recently, interest has developed in the allelopathic effects of seeds on the seeds and seedlings of other species (Suman et al. 2002; Qaderi et al. 2003; Rashid et al. 2005). However, the majority of seed allelopathy studies have focused on agroecosystems. The role of allelopathic seeds on natural vegetation process remains largely unknown.

L. virgaurea is often a dominant species in degraded alpine meadows, replacing communities dominated by *Kobresia capillifolia* or *Elymus nutans*. Grass abundance declines in degraded grasslands, but the underlying mechanisms driving this decline remain unclear (Xing 2009). Recent research has focused on the reproductive strategies and potential allelopathy of *L. virgaurea* as explanations for that species' dominance. *L. virgaurea* exhibits aggressive sexual (Ma et al. 2006a) and asexual (Wang et al. 2008) reproduction, but there is also evidence for allelopathic effects from volatile and aqueous leaf and root extracts on the germination and growth of native forb species (Ma et al. 2005, 2006b).

In the present study, we examine the potential of *L. virgaurea* for seed allelopathy. *L. virgaurea* germinates between mid- to late May, similar to the timing of most native grass species in Tibetan grasslands (Jia, unpublished data). Thus an allelopathic effect of *L. virgaurea* seeds on seed germination and seedling growth rates of other species may place *L. virgaurea* seedlings at a significant advantage. In this study we test whether the presence of *L. virgaurea* seeds or seed leachate negatively influence seed germination and root growth rates of common Tibetan native grass species.

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Materials and methods

Study site and species

The grasslands of the high altitude Tibetan Plateau are characterized by low temperatures and a short growing season. *Kobresia capillifolia*-dominated alpine meadows make up much of the usable rangeland. Rapid intensification of land use in the Tibetan Plateau has led to significant grassland degradation (Nan 2005). This degradation, particularly in the eastern parts of the plateau, is characterized by an increase in noxious forbs including *L. virgaurea*, *Stellera chamaejasme* and *Anemone rivularis*, which replace *K. capillifolia* and grasses. Three replicates of 100 dried seeds were weighed for measurement of seed mass of each species.

In the fall of 2008, we collected matured seeds of *L. virgaurea*, *Festuca sinensis*, *Agrostis gigantean*, *Bromus inermis* and *Elymus nutans* from natural communities in MaQu (N33°59', E102°00'), Gansu, China. The site is located in the eastern part of the Tibetan Plateau at 3,500 m above sea level. The natural vegetation is an alpine meadow normally dominated by *Kobresia capillifolia*, but following grazing dominated by *L. virgaurea* (Niu et al. 2009, 2010).

L. virgaurea is an herbaceous perennial species in the family Compositae. It reproduces clonally by rhizomes and through sexual reproduction. *L. virgaurea* produces several leaves from a very short stem at the vegetative stage, and develops a raceme of several capitula during flowering. The small seeds (dry seed mass = 16.6 mg) are wind dispersed achenes with a pappus. In previous studies we have found that *L. virgaurea* seedlings are shade-intolerant with a preference for bare and disturbed habitats (Wang et al. 2009).

E. nutans is a tall grass (80–130 cm) with flat large leaves (60–180 × 3–5 mm) spreading along the stem and relatively large seeds (dry seed mass = 3.85 mg). *F. sinensis* (40–70 cm tall) is often a subdominant with *E. nutans*. *F. sinensis* produces small seeds (dry seed mass = 8.5 mg). *B. inermis* is a tall (80–100 cm) grass with lanceolate leaves that can reproduce by either rhizomes or large seeds (dry seed mass = 2.4 mg). *B. inermis* has a strong ability to regrow following grazing. *A. gigantean* is a tall (90–150 cm) grass with flat leaves (17–30 cm × 3–8 mm) and spreads aggressively by rhizomes and produces very small seeds (dry seed mass = 0.90 mg).

Experimental design

The effects of *L. virgaurea* seed density on grass seed germination were tested using a factorial design with target species identity (*F. sinensis*, *A. gigantean*, *B. inermis* and *E. nutans*) and *L. virgaurea* seed density (0, 5, 10, 20, or 40 seeds) as fixed factors. Before germination, all seeds were surface sterilized with 5.25% sodium

hypochlorite solution for 15 min and then rinsed with distilled water. Each replicate consisted of a Petri dish with ten seeds from one of the four target grasses on two layers of filter paper in a Petri dish moistened with 3 ml of distilled water. The target grass seeds were placed at regular intervals on a grid and *L. virgaurea* seeds (if present) were placed regularly between the grass seeds. Four replicates were established for each treatment combination and the Petri dishes were placed randomly within a growth-chamber with a 16-h day, 8-h night cycle with temperatures of 20 and 5°C, respectively. To avoid competition for water during germination, we irrigated the filter paper with 5–6 drops of distilled water per dish in every day. Germination was determined by counting the number of germinated seeds at 24-h intervals over a 14-day period and expressed as total percent germination. Germination was deemed to occur only after the radicles had protruded beyond the seed coat by at least 1 mm. The root length of all germinated grass seeds was measured after 14 days.

The potential allelopathic effects of *L. virgaurea* seed leachate were tested using a factorial design with *L. virgaurea* leachate and target species as fixed factors. The leachate was extracted by soaking 1,000 mg of seeds of *L. virgaurea* in 100 ml of distilled water for 48 h, and the aqueous solution was filtered through filter paper. Ten surface sterilized seeds of each of the four target grass species were placed and germinated within the leachate solution and the distilled water (control), respectively. Four replicates were established for each treatment combination. The germination conditions were the same as the experiment of seed density. Germination rates and root growth were measured as in the seed density experiment. Mean time to germination (MTG) was calculated to represent germination rate for each grass species with or without leachate. $MTG(d) = \sum T_i N_i / S_i$, where T_i is the number of days, N_i is the number of germinated seeds on the i th day, and S_i is the total number of germinated seeds.

Data analysis

We analyzed the effects of *L. virgaurea* seeds and leachate of seeds on germination and root length of target species with a two-way ANOVA. Significance differences among treatments were identified using the S–N–K test. Percent germination was arcsine-transformed and MTG and root length were log-transformed to meet the assumptions of ANOVA. The root length data exclude non-germinated seeds. Statistical analyses were performed using SAS/STAT software, version 8.0 (SAS 2001).

Results

A significant interaction demonstrated that the grass species responded very differently to *L. virgaurea* seed

density (Table 1). *F. sinensis* and *A. gigantean* germination rates were reduced significantly by increasing densities of *L. virgaurea* seeds (Fig. 1a, b), while *B. inermis* and *E. nutans* were not affected (Fig. 1c, d). Root growth of all species declined with increased seed density, however, three species *F. sinensis*, *E. nutans* and *B. inermis* responded more strongly than *A. gigantean* (Fig. 2a–d).

Similarly, a significant interaction demonstrated that grass species responded very differently to leachate from *L. virgaurea* seeds (Table 2). Leachate significantly decreased germination rates of *F. sinensis* and *A. gigantean* ($F = 20.91$, $p = 0.0038$; $F = 31.10$, $p = 0.0014$), but not *B. inermis* and *E. nutans* ($F = 3.00$, $p = 0.1340$; $F = 2.53$, $p = 0.1628$). In contrast, leachate increased MTG in all of four grass species (Fig. 3), but to a greater extent in *B. inermis* and *E. nutans* than in *F. sinensis* and *A. gigantean*. The leachate also strongly influenced the root lengths of seedlings of test grass species (Table 2). The root lengths of *F. sinensis*, *A. gigantean*, *B. inermis* and *E. nutans* decreased by $78 (\pm 6)$, $68 (\pm 4)$, $64 (\pm 3)$ and $60\% (\pm 5\%)$ relative to their respective controls.

Discussion

In this study we demonstrate that *L. virgaurea* seeds can have allelopathic effects on both the germination and root growth rates of native grass species in a laboratory setting. We further demonstrate that this effect is likely driven by a water-soluble seed leachate. These allelopathic effects are potentially an important mechanism increasing *L. virgaurea* seedling survival and establishment rates in degraded grasslands. This allelopathic mechanism may also contribute to the dominance of *L. virgaurea* in degraded grasslands because the large numbers of seeds produced by this species may reduce the competition experienced by adult *L. virgaurea* plants (Ma et al. 2006a).

Numerous studies have found evidence for the existence of seed-to-seed allelopathic effects, but few of these studies have linked these seed allelopathic effects to vegetation processes in natural ecosystems. Seed-to-seed allelopathy can be caused by chemical groups including non-protein amino acids, phenolics, vanillic acid, polyethylene glycol, and flavonoids (Friedman and Waller

Table 1 Effects of seed density of *Ligularia virgaurea* on final percent germination and root length for four grass species

Source	Percent germination			Root length		
	<i>df</i>	SS	<i>F</i>	<i>df</i>	SS	<i>F</i>
Species	3	3.14	20.78***	3	7.22	1,289.86***
Seed density	4	1.68	8.31***	4	1.41	189.09***
Species \times seed density	12	1.63	2.70**	12	0.16	7.11***
Error	60	3.03		60	0.11	
Total	79	9.48		79	8.90	

** $p < 0.01$, *** $p < 0.001$

Fig. 1 The effects of seed density of *Ligularia virgaurea* on the mean percent germination (± 1 SD) of four grass species *Festuca sinensis* (a), *Agrostis gigantean* (b), *Bromus inermis* (c), and *Elymus nutans* (d). Significant differences ($p < 0.05$, located by S–N–K-tests) between means are denoted by different letters for each species

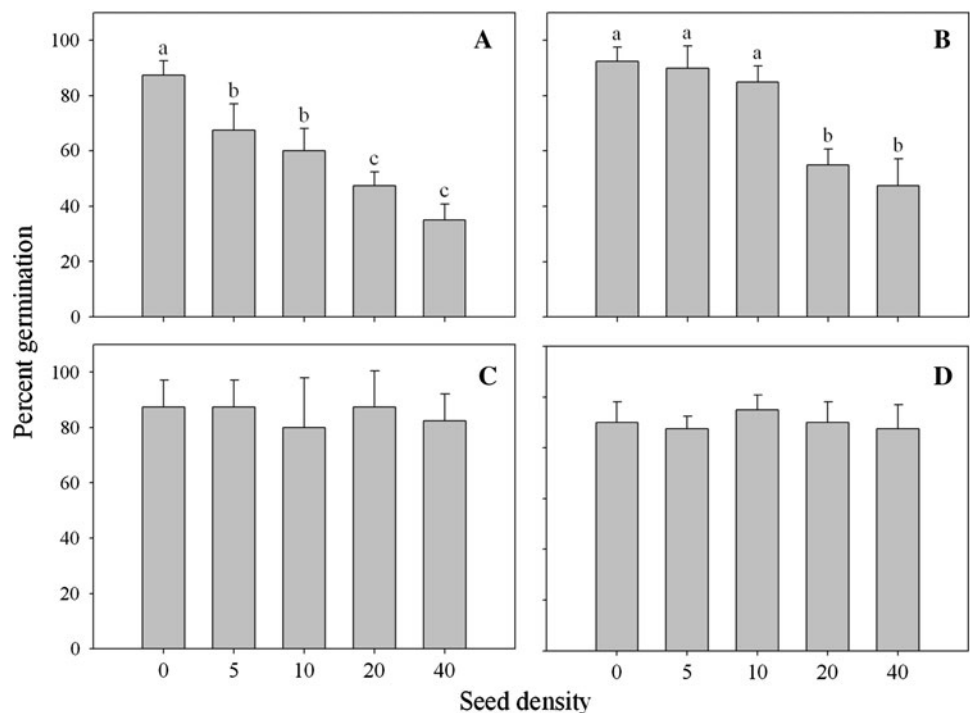


Fig. 2 The effects of seed density of *Ligularia virgaurea* on the mean root length (+1 SD) of four grass species *Festuca sinensis* (a), *Agrostis gigantea* (b), *Bromus inermis* (c) and *Elymus nutans* (d). Significant differences ($p < 0.05$, located by S–N–K-tests) between means are denoted by different letters for each species

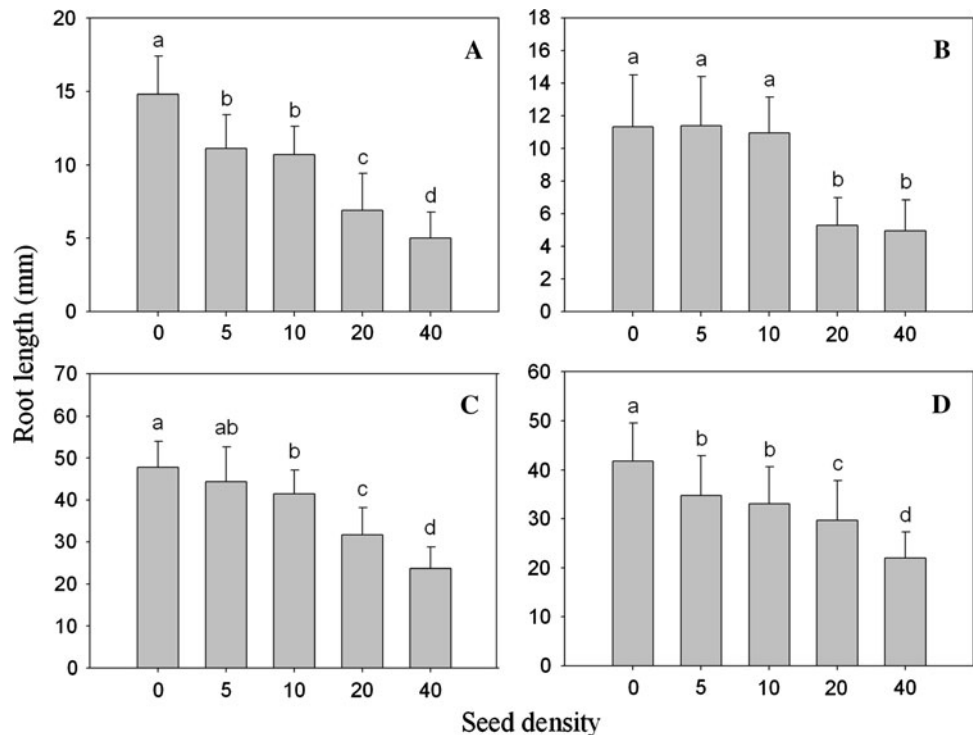


Table 2 Effects of *Ligularia virgaurea* seeds leachate on final percent germination, root length, and MTG for four grass species

Source	Percent germination			Root length			MTG		
	df	SS	F	df	SS	F	df	SS	F
Species	3	2.1	15.1***	3	2.6	231.4***	3	0.6	62.3***
Leachate	1	2.0	44.2***	1	2.0	539.1***	1	0.2	58.6***
Species × leachate	3	0.5	3.8*	3	0.07	6.7***	3	0.04	4.4*
Error	24	1.1		24	0.09		24	0.08	
Total	31	5.7		31	4.73		31	0.92	

* $p < 0.05$, *** $p < 0.001$

1983; Rashid et al. 2005; Kushima et al. 1998; Lateralra and Bazzalo 1999; Fottrell et al. 1964). Though the allelopathic compounds that *L. virgaurea* seeds release were not identified in this experiment, the potential allelopathy is clear from the inhibition of germination rates and root growth and increase of MTG. We predict that, for these species, *L. virgaurea* seeds will have significant negative effects on subsequent plant growth and establishment, and ultimately on density and biomass in the mature plant community. Similar allelopathic effects have been identified in other communities. For example, the dominant tussock grass *Festuca paniculata* uses allelochemicals to maintain dominance in subalpine grasslands by inhibiting growth of neighbor grasses (Viard-Crétat et al. 2009). Finally, interactions between grazing and allelopathy are likely very important in this community as *L. virgaurea* is not dominant in intact Tibetan grasslands (Niu et al. 2010). The removal of grass biomass by grazing releases resources and space for forb seeding establishment and growth (Gibson

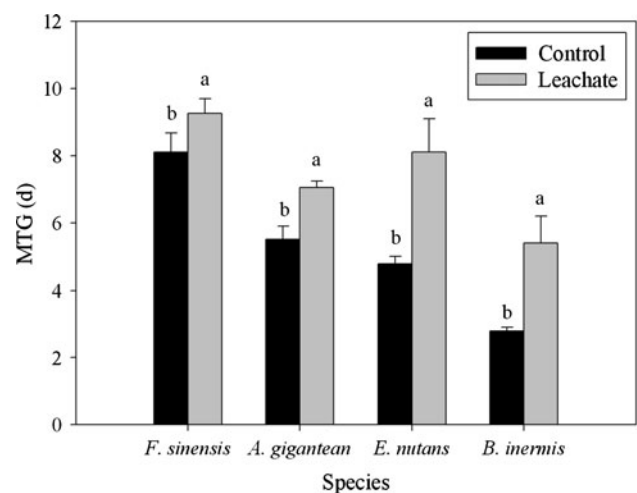


Fig. 3 Effects of leachate of *L. virgaurea* seeds on MTG (+1 SD) of four grass species. Significant differences ($p < 0.05$, located by S–N–K-tests) between means are denoted by different letters for each species

2009). Thus without an initial suppression of the grasses, seed allelopathy by *L. virgaurea* likely is not an important mechanism.

Our results show that the test species can be classified into two types according to their sensitivity to the toxicity of the presence of *L. virgaurea* seeds or leachate. A susceptible group, represented by *F. sinensis* and *A. gigantea*, had strongly inhibited germination and seedling growth, and for surviving seedlings increased mean germination time. A second group of less susceptible species, represented by *B. inermis* and *E. nutans*, had little direct inhibition of germination, but exhibited strongly reduced root development and increased MTG. This coincides with vegetation patterns in grazed grassland where *F. sinensis* and *A. gigantea* are relatively rare, and *B. inermis* and *E. nutans* more common (Niu et al. 2010). Other studies also indicate allelopathic effects are not exerted equally on all the species tested (Kil and Yun 1992; Chaves and Escudero 1997; Han et al. 2008). We consider the susceptibility of test species may be a function of seed size. The sensitive species *F. sinensis* and *A. gigantea* have a small seeds (dry seed mass = 8.5 and 0.9 mg, respectively) relative to the less sensitive species *B. inermis* and *E. nutans* (dry seed mass = 2.4 and 3.85 mg, respectively). The susceptibility of weed species to isothiocyanates (ITC) and benzoxazolin-2 (BOA) depends on seed size, with smaller seeded species generally more sensitive (Petersen et al. 2001; Belz and Hurlle 2005). Once seeds have germinated, however, it is the response of the new radicles to leachate that are most important. This may explain why only two species are greatly inhibited at the stage of germination while the radical growth of four all species were significantly suppressed.

Laboratory bioassays for allelopathy can be problematic (Inderjit and Nilsen 2003) as microbial degradation of many suspected allelopathic compounds occurs in native soils (Kamara et al. 1999). Additionally, the expression of allelopathic effects in the field can be mediated by stress factors, soil characteristics, and environmental conditions not tested in a laboratory bioassay (Alexa et al. 2008). Obviously, further field studies are needed to test this potential allelopathic effect before it can be used to fully explain the dominance of *L. virgaurea* in the degraded alpine grassland communities.

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