ΦYTON
REVISTA INTERNACIONAL DE BOTÁNICA EXPERIMENTAL
INTERNATIONAL JOURNAL OF EXPERIMENTAL BOTANY
FUNDACION ROMULO RAGGIO
Gaspar Campos 861, 1638 Vicente López (BA), Argentina
www.revistaphyton.fund-romuloraggio.org.ar

Allelopathic testing of *Pedicularis kansuensis* (Scrophulariaceae) on seed germination and seedling growth of two native grasses in the Tibetan plateau

Estudios alelopáticos de *Pedicularis kansuensis* (Scrophulariaceae) en la germinación y el establecimiento de plántulas de dos gramíneas nativas en el Tibet, China

Shang ZH[1,2] & SG Xu[1]

Abstract. *Pedicularis kansuensis* is a dominating poisonous weed, and it might have allelopathic effects on other native grasses in alpine meadows. An experiment was conducted to examine a range of concentrations of aqueous whole plant extracts (25, 12.5, 2.5, 1.25, 0.25 and 0.0 g/L) of *P. kansuensis*, prepared at the flowering stage on seed germination and seedling growth of two native grasses (*Poa pratensis* and *Elymus nutans*). High concentrations of aqueous extracts of *P. kansuensis* inhibited seed germination and seedling growth of *P. pratensis* ($p<0.05$). Most aqueous extracts of *P. kansuensis* had a stimulatory ($p<0.05$) effect on *E. nutans*. Our results suggest that the allelopathic ecology of *P. kansuensis* on other native grasses needs further experiments.

Keywords: Biological allelopathy; Aqueous extracts; Weeds; Native grasses; Alpine meadows.

Resumen. *Pedicularis kansuensis* es una maleza venenosa dominante que puede tener efectos alelopáticos en gramíneas nativas de la pradera alpina. Se condujo un estudio para evaluar los efectos de un rango de concentraciones de extractos vegetales acuosos (0; 0,25; 1,25; 2,5; 12,5; 25 g/L), preparados en el estadio de floración, a escala de plantas de *P. kansuensis* sobre la germinación y el crecimiento de plántulas de dos especies de gramíneas nativas: *Poa pratensis* y *Elymus nutans*. La germinación y el crecimiento de plántulas de *P. pratensis* fue inhibido ($p<0,05$) por altas concentraciones de los extractos acuosos de *P. kansuensis*. Sin embargo, la mayoría de los extractos acuosos de *P. kansuensis* tuvieron un efecto estimulante ($p<0,05$) sobre *E. nutans*. Nuestros resultados sugieren que futuros estudios deben seguir investigando los efectos de los extractos acuosos de *P. kansuensis* sobre las especies de gramíneas nativas.

Palabras clave: Alelopatía biológica; Extractos acuosos; Malezas; Gramíneas nativas; Praderas alpinas.

[1] International Centre for Tibetan Plateau Ecosystem Management, College of Pastoral Agriculture Science and Technology, Lanzhou University, Lanzhou 730020 China.
[2] Institute of Tibetan Plateau Research, Chinese Academy of Sciences, Beijing 10085 China.
Address Correspondence to: Shang Zhanhuan, *e-mail:* shangzhh@lzu.edu.cn
Recibido / Received 13.VI.2011. Aceptado / Accepted 23.VII.2011.

INTRODUCTION

Pedicularis kansuensis (Scrophulariaceae) is a very important medicinal plant, widely distributed over the alpine meadow of the Tibetan plateau (Zhao et al., 2005). However, it is also a highly invasive species, which produces volatile allelopathic compounds that might have an effect on other plants during its first year in the soil. *Poa pratensis* and *Elymus nutans* are the main local forage native grasses that are widely used to establish perennial pastures in the Tibetan plateau (Shi et al., 20006a,b). However, the allelopathic effects of *Pedicularis kansuensis* are not yet clear on other plant species.

Allelopathy appears to be an important competition mechanism in nature. The use of aqueous extracts appears to be closer to the natural effects of rainwater leaching than the use of organic solvents or other solutions to test its effects on seed germination and seedling growth of other plant species (Dietz et al., 1996; Jeffersion & Pennacchio, 2003; Chon et al., 2005). Therefore, research on the allelopathic effects of *P. kansuensis* on the native forage grasses of the Tibetian plateau is needed to initiate further studies on its biological characteristics in the alpine meadow. Our objective was to determine the potential allelopathic effect of *P. kansuens* on seed germination and seedling growth of *P. pratensis* and *E. nutans*.

MATERIALS AND METHODS

Plant and seed material. Plants of *Pedicularis kansuensis* Maxim (Scrophulariaceae) were collected during August 2007 from several sites at the field (34° 27´ N, 100° 12´ E; 3750-4000 m.a.s.l.) in a cold, dry region in the Tibetan Plateau, China. *Pedicularis kansuensis* is an annual species. After the growing season, the whole plant will fall on the ground, and the soil water will soak the whole plant body. Therefore, we took whole plants to do this study. The plants were taken to the laboratory at the flowering stage, and kept fresh in a refrigerator. Seeds of the grasses *Poa pratensis* Linn. and *Elymus nutans* Griseb. were collected in November 2006 from sown pastures in the same field where *P. kansuensis* was growing. These two forage grasses were chosen for study because they are often sown as pastures in the Tibetan plateau, where their populations decline greatly because of the invasion of *P. kansuensis*. Weight of 100 seeds of *P. pratensis* and *E. nutans* were 11.8 ± 0.17 mg and 374.9 ± 3.97 mg, respectively.

Extraction procedure. The extraction method followed Kato-Noguchi (2003), Jefferson & Pennacchio (2003), Chou et al. (2005) and Tang et al. (2008). Samples of the aboveground tissues (stems and leaves) of *P. kansuensis* were washed thoroughly with tap water and rinsed with distilled water. They were clipped using scissors leaving 1 to 2 cm. The clipped material was passed through a 1 mm screen before storage in a refrigerator at 2 °C. Each 100 g sample of plant tissues was extracted by soaking it in 4 L of distilled water at 24 °C during 24 h in a shaker. This gave an extract concentration equivalent to 25 g tissue/L. This was diluted with sterile distilled water to give final extract concentrations equivalent to 25, 12.5, 2.5, 1.25, and 0.25 g/L. All extracts were filtered through two layers of cheese cloth to remove fibers. Distilled water was used as the control treatment (CT).

Bioassay for germination. Seeds of the test species were sterilized in a 2% (w/v) solution of sodium hypochlorite for 15 min, and rinsed four times in distilled water. Fifty seeds of each species were sown on filter paper in Petri dishes, and allowed to germinate in an illuminated cultivation cabinet. The alpine conditions at an altitude of about 3800 m in the Tibetan plateau were simulated using a 12 h/12 h cycle of 5 °C dark/25 °C light. Three replicate Petri dishes of each treatment (including the control) were moistened with the appropriate concentration of extract. The moist conditions were maintained by adding 1 mL of the extract solution daily. Seedling emergence was recorded every 24 h until there was no further seedling emergence for 7 consecutive days. At this time, the shoot and root lengths were measured on seedlings, the germinated seeds counted, and the percentage germination calculated in all the treatments (Kato-Noguchi, 2003; Jefferson & Pennacchio, 2003; Tang et al., 2008).

Bioassay for growth. Seeds of the test species were allowed to germinate on filter paper in the dark at 25 °C for several days. Thereafter, 30 germinated seeds of each species were separately arranged on quartz sand in Petri dishes, and incubated at 25 °C for 10 days with artificial illumination (12 h light/12 h dark). The Petri dishes were irrigated with extract solutions, and the control with distilled water. Seedling shoot and root lengths were measured with a ruler in all treatments (Kato-Noguchi, 2003; Jefferson & Pennacchio, 2003; Tang et al., 2008).

Data analysis. The effect of extract solution on seed germination and seeding growth were calculated using the following parameters as reported by other researchers (Williamson & Richardson, 1988; Saxena et al., 1996; Jefferson & Pennacchio, 2003; Yan et al., 2006).

(1) Final germination rate *(Gr)* %:

$$Gr = \frac{\sum Gt}{Nt} \text{ x } 100\%$$

Gr is the percentage of emerged seedlings/total number of seeds sown; *Gt* is the seedling number at day t, *Nt* is the total number of seeds sown in the experiment.

(2) Germination speed index *(Gi)*

$$G_i = \sum \left({}^{G_t}/_{D_t} \right)$$

G_i is the seed germination speed of the species; G_t is seedling number at day t, D_t is the number of days when seedling numbers were recorded.

(3) Vigor index *(V_i)*

$$V_i = G_i \times S$$

Vi is the ability of seeds to germinate; *Gi* is the germination speed, *S* is the seedling length (cm).

(4) Response index *(RI)*

$$RI = 1 - \left({}^{C}/_{T} \right) \text{ (If } T > C\text{)}$$
$$RI = \left({}^{T}/_{C} \right) - 1 \text{ (If } T < C\text{)}$$

RI ranges from -1 to +1, with positive values indicating stimulation by the treatments and negative values indicating inhibition relative to the controls. The absolute value of *RI* ($\overline{RI}$) is the degree of inhibition and stimulation by the aqueous extracts.

A one-way analysis of variance was performed on the data. Probabilities less than 0.05 were considered significant. Significance of differences between individual means was determined using the LSD test.

RESULTS

Seed germination. Effects of extracts of *Pedicularis kansuensis* on seed germination in *Poa* pratensis are shown in Table 1. Analysis of variance of germination rate (Gr) showed no significant effect of extracts on seed germination, although when expressed relative to seeds on the control treatment, RI of Gr was negative. Inhibition by the extracts on *P. pratensis* germination speed began at concentrations of 2.5 g/L (RI<0) and significantly increased ($p<0.05$) with concentration. Seed vigor of *P. pratensis* was significantly reduced ($p<0.05$) by the extracts at concentrations in excess of 2.50 g/L. Effects of extracts of *P. kansuensis* on seed germination in *E. nutans* are shown in Table 2. Germination speed of seeds was not affected at low extract concentrations, but it was significantly reduced at 12.5 g/L compared with the CT.

Seedling growth. Growth of *P. pratensis* shoots was inhibited (RI<0) by *P. kansuensis* only when extract concentrations were higher than 12.50 g/L (Fig. 1). Although the lowest concentration of extract (0.25 g/L) significantly promoted root growth, extract concentrations above 1.25 g/L significantly inhibited root growth of seedlings.

There were no significant effects ($p>0.05$) of *P. kansuensis* extracts on shoot growth of *E. nutans* seedlings, but there was a stimulatory effect (RI>0) on root growth at concentrations above 2.5 g/L; the effect appeared to increase with greater concentrations of the extract (Fig. 2, Table 3).

Table 1. Effects of *Pedicularis kansuensis* aqueous extracts on seed germination rate (Gr) and germination speed (Gi), and viability (Vi) of seedlings in *Poa pratensis.*

Tabla 1. Efectos de extractos acuosos de *Pedicularis kansuensis* en la tasa (Gr) y velocidad (Gi) de germinación de las semillas, y la viabilidad (Vi) de plántulas de *Poa Pratensis.*

Aqueous extracts (g/L)	Gr	RI of Gr	Gi	RI of Gi	Vi	RI of Vi
CT	52.67 ± 0.67 a	-	9.00 ± 1.96 a	-	34.33 ± 7.54 a	-
0.25	42.67 ± 2.67 ab	-0.19	9.15 ± 1.68 a	0.02	28.60 ± 3.40 a	-0.17
1.25	46.67 ± 4.67 ab	-0.11	9.59 ± 1.43 a	0.06	31.59 ± 4.72 a	-0.08
2.50	42.67 ± 3.33 ab	-0.19	8.82 ± 1.87 a	-0.02	27.31 ± 7.85 a	-0.2
12.50	41.33 ± 3.53 b	-0.22	4.12 ± 0.61 b	-0.54	6.38 ± 1.49 b	-0.81
25.00	48.00 ± 5.03 ab	-0.09	5.07 ± 0.73 ab	-0.44	11.10 ± 2.86 b	-0.68

Different letters within a column indicate significant differences between treatment means for each of the measured parameters, using LSD at a significance of $p<0.05$.

Letras diferentes en una misma columna indican diferencias significativas entre tratamientos para cada uno de los parámetros medidos, usando LSD a un nivel de significancia de p<0,05.

Table 2. Effects of *Pedicularis kansuensis* aqueous extracts on seed germination rate (Gr) and germination speed (Gi), and viability (Vi) of seedlings in *Elymus nutans.*
Tabla 2. Efectos de extractos acuosos de *Pedicularis kansuensis* en la tasa (Gr) y velocidad (Gi) de germinación de las semillas, y la viabilidad (Vi) de plántulas de *Elymus nutans.*

Aqueous extract (g/L)	Gr	RI of Gr	Gi	RI of Gi	Vi	RI of Vi
CT	46.67 ± 5.46 b	-	4.55 ± 0.62 b	-	42.42 ± 3.00 b	-
0.25	71.33 ± 5.81 a	0.35	9.78 ± 1.16 a	0.53	85.98 ± 8.48 a	0.51
1.25	70.00 ± 1.15 a	0.33	10.34 ± 0.54 a	0.56	95.84 ± 8.71 a	0.56
2.50	56.67 ± 3.7 1 ab	0.18	9.43 ± 0.97 a	0.52	93.60 ± 14.04 a	0.55
12.50	66.67 ± 6.36 a	0.30	11.04 ± 0.77 a	0.59	101.41 ± 12.03 a	0.58
25.00	62.00 ± 5.03 a	0.25	8.83 ± 0.82 a	0.48	86.39 ± 8.78 a	0.51

Different letters within a column indicate significant differences between treatment means for each of the measured parameters, using LSD at a significance of p<0.05.
Letras diferentes en una misma columna indican diferencias significativas entre tratamientos para cada uno de los parámetros medidos, usando LSD a un nivel de significancia de p<0,05.

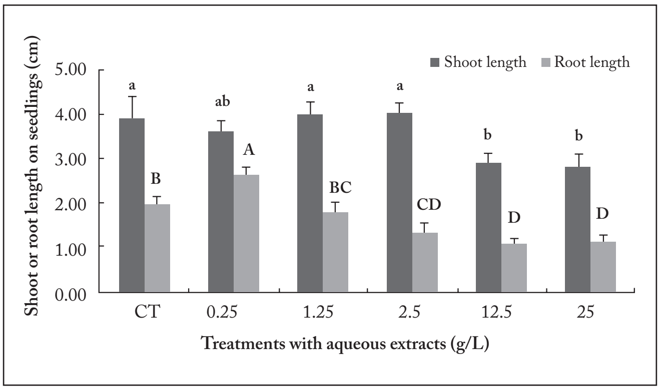

Fig.1 Effects of *Pedicularis kansuensis* aqueous extracts on seedling growth of *Poa pratensis*. Different lower case or capital letters indicate significant differences between concentrations for shoot length or root length, respectively. LSD was used at a significance level of p<0.05.
Fig. 1. Efectos de extractos acuosos de *Pedicularis kansuensis* en el crecimiento de plántulas de *Poa pratensis*. Letras minúsculas o mayúsculas diferentes indican diferencias significativas entre concentraciones para longitud de tallo o raíz, respectivamente. Se usó LSD a un nivel de significancia de p<0,05.

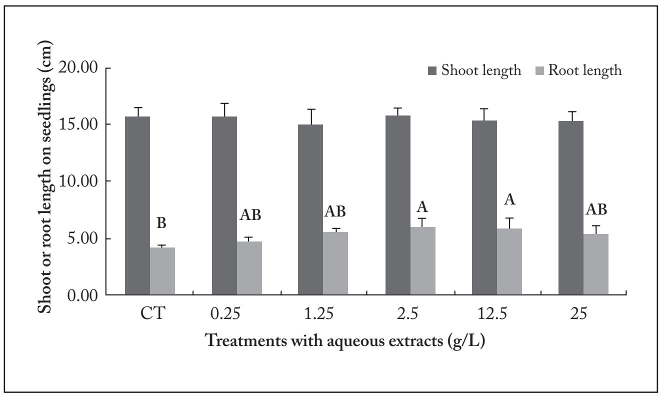

Fig. 2 Effect of *Pedicularis kansuensis* aqueous extracts on seedling growth of *Elymus nutans*. Different capital letters indicate significant differences between concentrations for root length. LSD was used at a significance level of p<0.05. Absence of letters on histograms corresponding to shoot length indicates no significant differences (p>0.05).
Fig. 2. Efectos de extractos acuosos de *Pedicularis kansuensis* en el crecimiento de plántulas de *Elymus nutans*. Letras mayúsculas diferentes indican diferencias significativas entre concentraciones para longitud de raíces. Se usó LSD a un nivel de significancia de p<0,05. La ausencia de letras sobre los histogramas correspondientes a la longitud de tallos indica la ausencia de diferencias significativas (p>0,05).

Table 3. Inhibition or stimulation (RI) index of aqueous extracts on grass seedling growth.
Tabla 3. Indice de inhibición o estimulacion de extractos acuosos en el crecimiento de plántulas de gramíneas.

Aqueous extracts (g/L)	*Poa pratensis*		*Elymus nutans*	
	Shoot length	Root length	Shoot length	Root length
0.25	-0.08	0.24	0.00	0.12
1.25	0.02	-0.09	-0.05	0.25
2.50	0.03	-0.33	0.01	0.31
12.50	-0.26	-0.47	-0.02	0.32
25.00	-0.28	-0.43	-0.03	0.24

DISCUSSION

Our experiments showed that aqueous extracts of *P. kansuensis* had an inhibitory effect on seed germination (including germination rate, speed and vigor) of *P. pratensis* at high concentrations. However, they showed stimulatory effects on *E. nutans* seeds. Thus the effect of the extracts on the study species was dependant on their concentration (Jefferson & Pennacchio, 2003). While some studies reported positive effects of weed extracts on grasses, some Chinese publications only showed negative effects of weed allelopathy on grasses (Zhang et al., 1989; Bai & Zhang, 1994; Ma et al., 2006b).

Seed size of *E. nutans* is more than 30 times larger than that in *P. pratensis.* Also, seed coat is thicker in *E. nutans* than in *P. pratensis*. Differences in seed resistance between species may explain why the low concentration extract of *P. kansuensis* did not have enough solvent action on *E. nutans* seed fur to give a positive effect on the seed. Alternatively, the very small seeds of *P. pratensis* make contact easily with the aqueous extract, so that even low concentrations can cause an immediate negative effect. Our experiment suggests that *P. kansuensis* extract concentrations higher than 25 g/L may produce inhibitory effects on *E. nutans.* This is because the stimulatory extract effects at a concentration of 25 g/L were less than those at the 12.5 g/L extract. However, the concentration of the allelopathic material may be very low under natural conditions in the Tibetan plateau.

There is some uncertainty regarding the effects of different extract concentrations in allelochemical tests on seed germination and seedling growth. This is the result of the contrasting information reported in many trials (Saxena et al., 1996; Kato-Noguchi, 2003; Chon et al., 2003; Jefferson & Pennachhio, 2003). Entire plants of *P. kansuensis* are buried in the soil at the second year of its life cycle; since there is much water in alpine grasslands in spring, whole plants of *P. kansuensis* will be soaked in soil water, at the same time that seed germination and seedling growth occur in other species. There are no data on the concentration of aqueous extracts in the soil, but concentrations from 25 to 80 g/L have been used in allelopathic experiments (Saxena et al., 1996). They were obtained by crushing plants in receptacles. In our experiment, we considered that aqueous solution concentrations of less than 25.0 g/L, and the use of *P. kansuensis* in allelopathic experiments, would be close to a naturally occurring situation. This considers the amount of rain water in the soil during seedling emergence in the alpine meadow. The effects of *P. kansuensis* on *E. nutans* are therefore complex, and indicate that the effect of weeds on grasses is a complicated process.

ACKNOWLEDGEMENTS

This research was supported by the National Natural Science Foundation of China (No. 41171417; 30730069) and Youth Cross-Science Foundation of Lanzhou University (No. LZU-JC2007008). We thank Prof. Malcolm Gibb (Formerly of The Institute of Grassland and Environmental Research, UK) for suggestions and editing the manuscript.

REFERENCES

Bai, X.F. & B.C. Zhang (1994). Study of staple composition of *Ajania tenuifolia* oil on inhibition mechanism seeding initial growth of *Elymu nutans*. *Acta Ecologica Sinica* 14: 223-224.

Chon, S.U., H.G. Jiang, D.K. Kim, Y.M. Kim, H.O. Boo & Y.J. Kim (2005). Allelopathic potential in lettuce (*Lactuca sativa* L.) plants. *Scientia Horticulture* 106: 309-317.

Dietz, H., T. Steinlein, P. Winterhalter & I. Ullman (1996). Role of allelopathy as a possible factor associated with the rising of dominance of *Bunias orientalis* L. (Brassicaceae) in some native plant assemblages. *Journal of Chemical Ecology* 22: 1797–1811.

Jefferson, L.V. & M. Pennacchio (2003). Allelopathic effects of foliage extracts from four chenopodiaceae species on seed germination. *Journal of Arid Environments* 55: 275-285.

Kato-Noguchi, H., (2003). Assessment of allelopathic potential of shoot powder of lemon balm. *Scientia Horticulturae* 97: 419-423.

Ma, R.J., M.L. Wang, K. Zhao, S.J. Guo, Q.F. Zhao & K. Sun (2006b). Allelopathy of aqueous extract from *Ligularia virgaurea*, a dominant weed in psychro-grassland on pasture plants. *Chinese Journal of Applied Ecology* 17: 845-850.

Saxena, A., D.V Singh & N.L. Joshi (1996). Autotoxin effects pearl millet aqueous extracts on seed germination and seedling growth. *Journal of Arid Environments* 33: 255-260.

Shi, J.J., Y.S. Ma, L.Y. Wang, Q.M. Dong, Q.Y. Li, Y.F. Li & D.Z. Lai (2006b). Evaluation of adaptability of 10 *Poa* forages on artificial vegetation in 'black soil type' degraded grassland. *Chinese Qinghai Journal of Animal and Veterinary Science* 36: 14-16.

Shi, J.J., L.Y. Wang, Y.S. Ma, Q.M. Dong & L.Y. Wang (2006a). Evaluation of adaptability of 3 *Elymus* forages on artificial vegetation in 'black soil type' degraded grassland. *Chinese Qinghai Journal of Animal and Veterinary Science* 36: 4-6.

Williamson, G.B. & D. Richardson (1988). Bioassays for allelopathy: measuring treatment responses with independent controls. *Journal of Chemical Ecology* 14: 181-187.

Yan, Q.C., W.M. Hu & W.J. Song (2006). Principle and Methods of Seed Vigour Test. Beijing: China Agriculture Press.

Zhang, B.C., X.F. Bai, L.H. Gu & R.D. Zhen (1989). Study on allelopathy and natural degeneration phenomena of artificial grassland on alpine meadow. *Acta Ecologica Sinica* 9: 115-120.

Zhao, X.P., X.M. Wu & X. Su (2005). Study on the resource surveying researching and exploiting of *Pedicularis kansuensis* maxim in Qinghai province. *Qinghai Prataculture* 14: 44-48.