

# Chemical effects of a dominant grass on seed germination of four familial pairs of dry grassland species

Eszter Ruprecht<sup>1\*</sup>, Tobias W. Donath<sup>2</sup>, Annette Otte<sup>2</sup> and R. Lutz Eckstein<sup>2</sup>

<sup>1</sup>Department of Taxonomy and Ecology, Babeş-Bolyai University, Republicii street 42, RO-400015 Cluj Napoca, Romania; <sup>2</sup>Department of Landscape Ecology and Resource Management, Interdisciplinary Research Centre, Justus-Liebig-University Gießen, Heinrich-Buff-Ring 26-32, DE-35392 Gießen, Germany

## Abstract

Community composition and ecosystem processes during succession may be driven partly by traits of plant species that attain dominance. Here, we addressed the hypothesis that *Stipa pulcherrima*, the dominant grass of abandoned continental grasslands, controls seedling recruitment of co-occurring species through chemical effects of its litter. Eight species with successful and unsuccessful recruitment under field conditions were selected (four familial pairs) to study experimentally the effects of leaf leachate under four temperature regimes. Since fungi developed in leachate-treated Petri dishes, in another experiment seeds were surface sterilized to remove confounding effects of fungi on recruitment. Leachate affected various stages of seedling recruitment: it significantly reduced seed germination (by 33–94%) and radicle elongation, and it delayed germination of seedlings of all species. In two families, species with unsuccessful field recruitment were more negatively affected than the successful ones. In a third family, the species with successful recruitment was more negatively affected, and in the fourth there were no differences. Similar germination responses after exclusion of fungi through seed-surface sterilization suggested that leachate was responsible for the observed effects on recruitment. Besides other traits and physical/microclimatic effects of accumulating litter, *S. pulcherrima* influences plant community dynamics and may potentially affect ecosystem processes through its secondary compounds.

**Keywords:** allelochemicals, allelopathy, community dynamics, fungi, radicle length, seedling establishment, *Stipa* grassland, *Stipa pulcherrima*

## Introduction

Community assembly during succession may be regulated by: (1) environmental conditions and resources; (2) chance events; and (3) biotic interactions (Weiher and Keddy, 1999). Through the traits of dominant species, the outcome of biotic interactions (herbivory, competition, facilitation) may, in turn, feed back on abiotic conditions and resource availability, and strongly determine the further development of the system. One model system that is currently undergoing great change is European semi-natural grassland, which carries an exceptionally high diversity of plants (e.g. Ellenberg, 1988; Korneck *et al.*, 1998). Diversity and composition of these ecosystems evolved under the prevailing site conditions (Vandvik and Birks, 2002), but also in response to continuous human activity over millennia (Ellenberg, 1988; Pärtel *et al.*, 1996). Consequently, their characteristic species are adapted to human land use, and grassland diversity is strongly related to past and current management practices (Waldhardt and Otte, 2003; Wellstein *et al.*, 2007). With agricultural abandonment and intensification, plant diversity in semi-natural grassland communities changes dramatically (Korneck *et al.*, 1998; Luoto *et al.*, 2003; Mitchley and Xofis, 2005; Pykälä *et al.*, 2005; Wellstein *et al.*, 2007; Enyedi *et al.*, 2008). The resulting decrease in species numbers is mainly due to: (1) increasing cover and biomass of a few highly productive species, which exert strong competitive effects on subordinate species; and (2) the accumulation of dead plant remains, i.e. litter, which interferes with seed germination and seedling establishment (Bosy and Reader, 1995; Virágh and Bartha, 1996; Bakker and Berendse, 1999; Kahmen *et al.*, 2002; Moog *et al.*, 2002). Litter may affect seed germination by its effects on microclimate, by acting as a mechanical barrier or through chemical effects (Facelli and Pickett, 1991; Bosy and Reader, 1995; Jensen and Gutekunst, 2003; Eckstein and Donath, 2005). Since these effects are litter-type specific, the dominant plant species may

\*Correspondence

Fax: + 40 264431858

Email: ruprecht@grbot.ubbcluj.ro

exert a strong influence on germination and succession (Myster, 2006; Quested and Eriksson, 2006; Quested *et al.*, 2007; Donath and Eckstein, 2008), but also on ecosystem processes (Wardle *et al.*, 1998).

The regeneration of plants from seeds is a process that can be broken down into several conditional components, such as reproductive fertility and seed production, seed viability, germination, seed resource-based initial seedling growth (radicle elongation, leaf expansion), autotrophic seedling growth and establishment. As such, it is a particularly vulnerable stage in the life cycle of many plant species, and the suppression of seedling recruitment can have serious consequences for population viability and species diversity (e.g. Tilman, 1993). In this context allelopathy, i.e. the leaching or volatilization of phytotoxins from plant tissues, is considered as one possible mechanism acting on seed germination (Baskin and Baskin, 2001), but also on seedling establishment (Schlatterer and Tisdale, 1969; Chang-Hung and Chiu-Chung, 1975; Werner, 1975; Bosy and Reader, 1995), and plant growth and distribution (del Moral and Cates, 1971; Rice, 1972; Newman and Rovira, 1975). Chemicals involved are generally secondary metabolites, mainly simply structured, low molecular weight compounds, such as coumarins, terpenoids, phenolics or tannins (Rice, 1984; Harborne, 1993). However, since there are close links between plant secondary compounds, palatability, litter quality and decomposition (Grime *et al.*, 1996), allelochemicals may also affect other components and functions of the ecosystem, and thus play an important role in ecosystem regulation (Wardle *et al.*, 1998; Mazzoleni *et al.*, 2007).

Biochemical interactions differ in their strength and may differentially affect the competitive vigour of dominant and subordinate species. Hence, to understand these interactions and the potential influence of dominant species on ecosystem processes, it is necessary to identify plant species in a community with strong allelopathic effects on other species (Chang-Hung and Chiu-Chung, 1975; Anaya and del Amo, 1978; Anderson *et al.*, 1978), but it is equally important to reveal the differential susceptibility of community constituents to these toxins (Datta and Sinha-Roy, 1975; Wardle *et al.*, 1993).

Preliminary results of a manipulative field experiment on abandoned grasslands from the Transylvanian lowlands of Romania (Ruprecht *et al.*, in preparation) suggested that secondary compounds of the dominant grass may, among other factors, drive community assembly and succession through strong negative effects on the recruitment of a number of co-occurring plant species. These species-rich, dry, steppe-like grasslands occur on steep south-facing slopes with eroded carbonated chernozemic soils on clayish or marly substrate, and are dominated by feather-grass (*Stipa*) species. The main consequences of long-term

abandonment of these grasslands are: (1) strong dominance by *Stipa pulcherrima*; (2) litter accumulation; (3) vegetation composition changes; and (4) decrease of plant species diversity (Enyedi *et al.*, 2008). Under several experimental treatments, consisting of litter and/or biomass removal, establishment success of certain species was very low (see Table 1; Ruprecht *et al.*, in preparation), although there were no indications of seed or microsite limitation, since plants flowered abundantly, and various types of potential safe sites for germination and establishment were created by the treatments. Another group of dry grassland species with abundant flowering and seed production established successfully under the same experimental conditions (see Table 1). Therefore, we investigated experimentally the possible chemical effects of the dominant species, *Stipa pulcherrima*, on the germination of seven co-occurring dry grassland species and on itself at two constant and two fluctuating temperature regimes. Previous studies addressing chemical effects of litter have either used one constant (e.g. Anaya and del Amo, 1978; Bosy and Reader, 1995) or one fluctuating temperature regime (e.g. Rice, 1972; Norby and Kozłowski, 1980), whereas our experimental set-up allowed us to address the interaction between litter leachate and temperature across a range of conditions. Our study attempts to link potential chemical effects at the population level with known transformations in ecosystem properties in a dry grassland community as a result of grazing abandonment.

Our main hypotheses were that: (1) *Stipa* leachate reduces percentage germination, delays germination, and interferes with radicle elongation, thus exerting strong negative effects on the regeneration through seeds of the eight test species; and that (2) there are species-specific differences in the response of these various components of recruitment to leachate. (3) We hypothesized that the magnitude of the effect of *Stipa* leachate is related to the germination success under field conditions, i.e. the negative effects are more pronounced in species with unsuccessful than in those with successful field recruitment.

## Materials and methods

### Species selection

Eight grassland species, which are typical constituents of dry steppe-like grasslands of the Transylvanian Lowland (Romania), were selected for laboratory seed germination experiments, based on the results of previous field experiments (Table 1). The species set consisted of four familial pairs (*Asteraceae*, *Fabaceae*, *Rubiaceae*, *Poaceae*), where one species of each pair was from the group with successful establishment and the other from the group with unsuccessful establishment

**Table 1.** Species abbreviations (Abbr.), field germination, establishment success under field conditions, family, diaspore (seed) mass, and seed viability of the eight dry grassland species selected for the germination experiments

Species selected*	Abbr.	Field germination (seedlings m <sup>-2</sup> )	Success	Family	Diaspore mass (mg)	Seed viability (%)
<i>Serratula radiata</i> (WaldS. & Kit.) Bieb.	Sr	0	–	Asteraceae	2.53	80
<i>Jurinea mollis</i> (L.) Rchb. subsp. <i>transylvanica</i> (Spreng.) Hayek	Jm	6.2	+	Asteraceae	3.45	70
<i>Medicago sativa</i> L. subsp. <i>falcata</i> (L.) Arcang.	Ms	1.5	–	Fabaceae	1.11	72
<i>Dorycnium pentaphyllum</i> Scop. subsp. <i>herbaceum</i> (Vill.) Rouy	Dp	43.3	+	Fabaceae	1.56	66
<i>Galium glaucum</i> L.	Gg	0	–	Rubiaceae	1.09	70
<i>Asperula cynanchica</i> L.	Ac	42.8	+	Rubiaceae	0.63	56
<i>Stipa pulcherrima</i> C. Koch	Stp	2.5	–	Poaceae	24.29	92
<i>Stipa capillata</i> L.	Stc	28.7	+	Poaceae	5.14	98

Field germination is the sum for the 2 years of field observations (2006, 2007) of the cumulative number of seedlings counted in two dry grassland sites (Suatu, Puini), expressed as seedlings m<sup>-2</sup>. Data on seed mass are the results of measurements of 500 (5 × 100) seeds per species. Seed viability of 50 (2 × 25) seeds per species was tested with a 1% tetrazolium chloride solution.

\* Plant nomenclature follows *Flora Europaea* (Tutin *et al.*, 1964–1980).

under field conditions (Table 1). Family pairs were used to obtain a phylogenetically balanced data set.

### Seed collection

Propagules of the eight species (called seeds from here on) were collected in bulk from autochthonous populations in dry steppe-like grasslands in the Transylvanian Lowland. Seed collection was carried out between May and August 2007, depending on the time of seed ripening, and included at least 100 different plant individuals from five sites each. Seeds were dry-stored in darkness at room temperature (*c.* 20°C) until the start of the experiments in autumn 2007.

### Germination experiment 1

The germination experiment was performed in climate chambers (Rumed, Rubarth Apparate GmbH, Laatzen, Germany) at the Department of Landscape Ecology and Landscape Planning, Gießen University, Germany. Fifty seeds of each species were spread on a double layer of filter paper in sterile Petri dishes. A total of 40 dishes per species were prepared, randomly distributed into two groups to test for chemical effects of *Stipa pulcherrima*. One group was watered with a leachate prepared from leaves of *S. pulcherrima*, whereas the other group received distilled water (control). Leaves of *S. pulcherrima* were collected in the middle–late phase of the vegetation period (July and August 2007) from two grassland sites (Suatu, Puini) and dried at room temperature. The leachate was prepared by soaking 344.67 g of dry leaves in 3300 ml of distilled water for 48 h. These amounts of leaves and water (0.1044 g leaves per ml water) correspond to the

average litter quantity in the field (470 g m<sup>-2</sup>) and the quantity of rain water of an average precipitation event (4.51 m<sup>-2</sup>), respectively (Cluj Napoca Meteorological Station). In the leachate and the control treatments, each sample was watered with 10 ml of the leachate solution or 10 ml of distilled water, respectively, once at the start of the experiment. The osmotic potential (Osmomat 030, Gonotec GmbH Berlin, Germany) and pH (Lab 860, Schott Instruments, Mainz, Germany) of the leachate were assessed, as these characteristics may influence seed germination.

The 20 dishes of each leachate treatment group were randomly distributed to the four temperature treatments, *i.e.* two constant (10 and 20°C) and two diurnally fluctuating temperatures (5/15°C and 10/25°C), and incubated in climate chambers. Five Petri dishes of each species were put together into a climate chamber and sealed to reduce evaporation. The light regime simulated spring day conditions with 12 h of light and 12 h of darkness.

The experiment was comprised of the following factors: *species* [factor levels (*k*) = 8], nested within *field establishment success* (*k* = 2), *temperature regime* (*k* = 4), and *leachate* (*k* = 2). Each combination of factors was replicated five times. Germinated seeds were counted and removed once every week for 6 weeks. A seed was considered germinated when the radicle was beginning to protrude from the seed coat. At each counting occasion, the radicle length of a maximum of five randomly chosen seedlings was measured using a millimetre paper. As dependent variables we used: (1) cumulative germination, calculated as the sum of germinated seeds over 6 weeks in relation to the total number of seeds; (2) the week of maximum germination; and (3) the radicle length of seedlings. Since in our experiment fungi developed in high abundance, especially in samples watered with

leachate, we visually estimated the cover of developing fungi in the dishes to the nearest 5% and determined fungal genera (or higher-order groups).

### Germination experiment 2

To separate the possibly confounding effects of leachate and fungi on seed germination, we carried out an additional experiment where we tried to exclude fungi to test the effect of leachate alone. The experimental set-up was analogous to that mentioned above, with the exception that seeds were surface sterilized by application of NaOCl (3% active chlorine), and the leachate was sterile-filtered to exclude spores of fungi and bacteria. We used one diurnally fluctuating temperature regime of 5/15°C (12/12h), which had proved to be an optimal germination temperature regime for most of the study species in the first experiment. The measures successfully prevented fungal growth in the Petri dishes containing *Jurinea mollis*, *Medicago sativa*, *Dorycnium pentaphyllum*, and *Asperula cynanchica* during the first 3 weeks of germination. Seedlings were counted and removed once every week.

### Data analysis

Data on cumulative germination, radicle length, week of maximum germination and fungal abundance were

analysed using a four-factorial hierarchical general linear model analysis of variance. The factor *species* was nested within *field germination success* (Table 1), and all factors were considered fixed. In a combined analysis, we used data on cumulative germination after 3 weeks for those four species that showed no fungal growth after the sterilization treatment. Here, we used a three-factorial general linear model analysis of variance with the factors *species*, *leachate* and *seed surface sterilization*, i.e. sterilization (no effect of fungi) versus no sterilization (seeds infected by fungi). Arcsine or square root transformation was used to improve homogeneity of variances. Tukey's HSD test was applied for *a posteriori* testing of multiple means (Quinn and Keough, 2002). All analyses were done using Statistica, version 6.0 (StatSoft Inc., Tulsa, Oklahoma, USA).

## Results

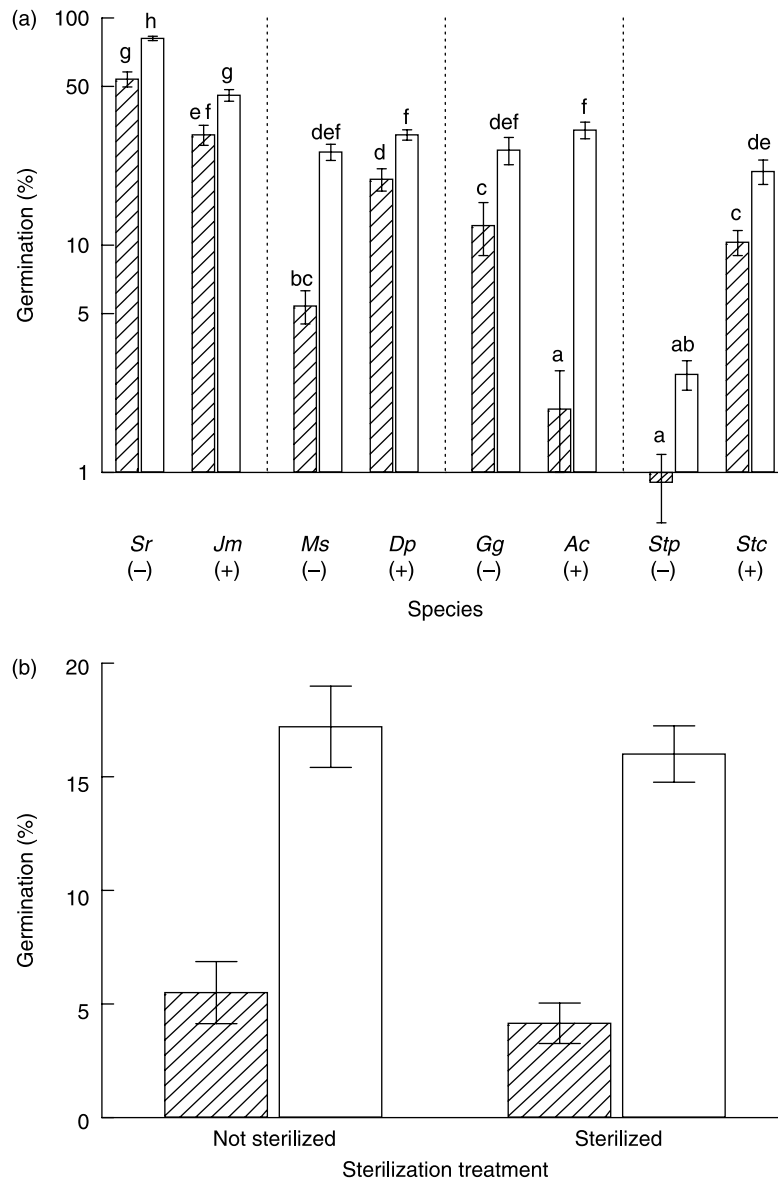
### Seed germination

Cumulative percentage germination across temperatures varied significantly among species (Table 2, Fig. 1a) and ranged between 1.8% (*S. pulcherrima*) and 67.6% (*S. radiata*). Under these experimental conditions, there were no general significant differences between species that showed successful or unsuccessful germination in the field. However, leachate significantly and strongly reduced

**Table 2.** Effects of species identity, establishment success under field conditions, temperature and leachate on percentage germination, week of maximum seed germination, and radicle length

Source of variation	Germination				Week of maximum germination				Radicle length			
	df	MS	<i>P</i>	vc (%)	df	MS	<i>P</i>	vc (%)	df	MS	<i>P</i>	vc (%)
Intercept	1	70.23	<0.0001		1	2619.18	<0.0001		1	1372.13	<0.0001	
Species (success)	6	3.12	<0.0001	61.40	6	35.33	<0.0001	22.07	4	4.33	<0.0001	6.66
Success	1	0.01	0.4808	0.02	1	4.72	0.0258	0.49	1	5.03	<0.0001	1.93
Temperature	3	0.03	0.0357	0.33	2	6.29	0.0014	1.31	3	16.32	<0.0001	18.82
Leachate	1	4.55	<0.0001	14.94	1	260.17	<0.0001	27.09	1	101.77	<0.0001	39.12
Species (success) × Temperature	18	0.09	<0.0001	5.15	12	2.05	0.0126	2.55	12	0.73	0.0013	3.35
Species (success) × Leachate	6	0.22	<0.0001	4.35	6	8.06	<0.0001	5.04	4	1.22	0.0010	1.88
Temperature × Leachate	3	0.05	0.0038	0.53	2	3.74	0.0196	0.78	3	5.59	<0.0001	6.44
Success × Leachate	1	0.01	0.5341	0.02	1	0.08	0.7698	0.01	1	0.79	0.0794	0.30
Success × Temperature	3	0.08	0.0003	0.75	2	0.31	0.7214	0.06	3	1.13	0.0049	1.30
Success × Leachate × Temperature	3	0.11	<0.0001	1.08	2	1.91	0.1333	0.40	3	0.23	0.4380	0.27
Species (success) × Temperature × Leachate	18	0.03	0.0019	1.62	12	2.09	0.0106	2.61	12	0.41	0.0872	1.91
Error	256	0.01		9.82	383	0.94		37.59	184	0.25		18.00

Data are the results of a four-way hierarchical ANOVA with species nested within establishment success. Temperature = constant temperatures: 10°C, 20°C, fluctuating temperatures: 5/15°C and 10/25°C. Data on relative germination were arcsine-transformed, and data on fungal abundance were square root-transformed before analysis. Owing to missing data, *S. pulcherrima* and *A. cynanchica* were excluded from the analysis of radicle length, and in the analysis of the week of maximum seed germination, the temperature level 20°C had to be omitted. Abbreviations: df, degrees of freedom; MS, mean sum of squares; vc (%), percentage of the total variation explained.

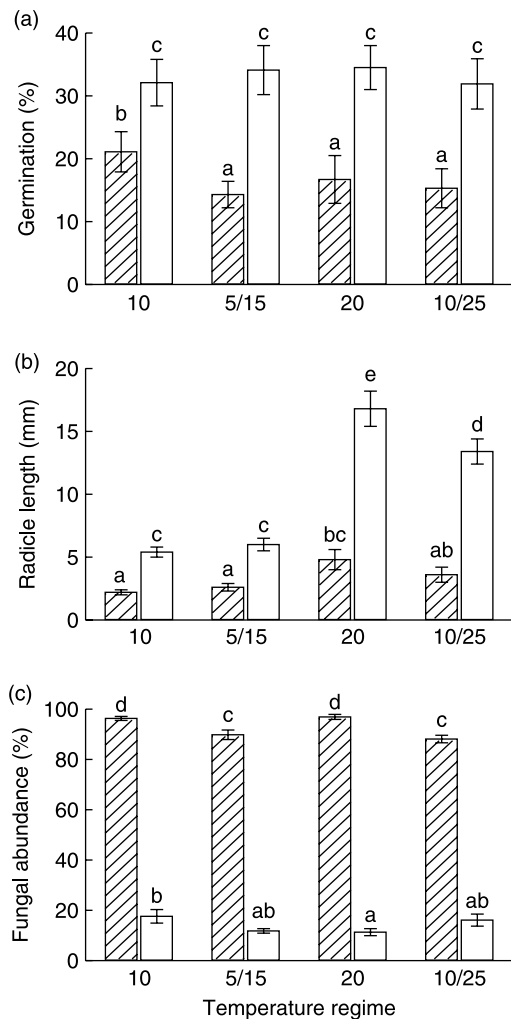


**Figure 1.** Percentage seed germination in Petri dishes treated with *Stipa pulcherrima* leaf leachate (hatched bars) and distilled water (white bars), (a) across temperature regimes after 6 weeks for all eight species and (b) pooled germination of surface sterilized and non-sterilized seeds of four species (*Jm*, *Ms*, *Dp* and *Ac*) after 3 weeks. For species abbreviations, see Table 1. Data are means  $\pm$  SE ( $n = 20$ ). In (a) dotted lines separate family pairs; species with successful and unsuccessful field germination are denoted by (+) and (-), respectively. Note logarithmic y-axis. Means sharing the same letter are not significantly different ( $P > 0.05$ ; Tukey's HSD). Panel (b) shows a comparison of data from the first and second experiment. In the latter, fungi were excluded through seed surface sterilization. The figure depicts the non-significant interaction between the effect of leachate and the presence of fungi ( $F_{1,64} = 0.01$ ,  $P = 0.92$ ).

percentage germination (Figs 1a and 2a); among species, germination was reduced by between 33 and 94% as compared to controls. This factor explained 14.9% of the total variation (Table 2).

In both the *Fabaceae* and *Poaceae*, the unsuccessful field germinator was more strongly affected by leachate than the successful species, whereas this

pattern was reversed in the *Rubiaceae*. For both *Asteraceae* species, the germination response did not differ between the successful and the unsuccessful field germinator. In comparison with the leachate effect, main temperature effects on final germination percentages were rather weak (Fig. 2a, Table 2). However, the effect of leachate was significantly lower



**Figure 2.** (a) Percentage germination, (b) radicle length and (c) fungal abundance in Petri dishes treated with *Stipa pulcherrima* leaf leachate (hatched bars) and distilled water (white bars) at different temperature regimes across species. Data are means  $\pm$  SE [in (a) and (b):  $n = 26$ – $30$ ; in (c):  $n = 40$ ]. Means sharing the same letter are not significantly different ( $P > 0.05$ ; Tukey's HSD on the temperature  $\times$  leachate interaction). *S. pulcherrima* and *A. cynanchica* were excluded from the analysis of radicle length because of missing values at certain temperatures.

at 10°C constant temperature than in the temperature regimes with higher daytime temperatures (Fig. 2a).

Strong leachate effects on germination were confirmed in the combined analysis of data on cumulative germination after 3 weeks from both experimental set-ups. Germination was significantly reduced ( $F_{1,64} = 274.39$ ,  $P < 0.0001$ ) by the application of leachate (Fig. 1b). The factor *leachate* alone explained 49.4% of the total variation. In contrast, there was neither a significant main effect of

sterilization ( $F_{1,64} = 3.2$ ,  $P > 0.08$ ) nor a leachate  $\times$  sterilization interaction ( $F_{1,64} = 0.01$ ,  $P = 0.92$ ). These results indicate that leachate, but not fungal effects, were responsible for significantly reduced cumulative germination in experiment 1.

### Timing of germination

Although the week of maximum germination also differed among species (Table 2), a large part of the variation (27.1%) was accounted for by the main leachate effect alone. The application of leachate at the start of the experiment significantly delayed the germination peak of the species by about 1.5 weeks.

### Radicle length

In contrast to cumulative germination, there was a strongly significant effect of temperature on radicle length ( $F_{3,184} = 64.1$ ,  $P < 0.0001$ ; Fig. 2b), which alone explained 18.8% of the variance (Table 2). Leachate significantly reduced radicle length ( $F_{1,184} = 399.8$ ,  $P < 0.0001$ ; explained variance: 39%). In addition, there was a significant temperature  $\times$  leachate interaction ( $F_{3,184} = 21.9$ ,  $P < 0.0001$ ), indicating that the negative effect of leachate in comparison to controls was stronger at higher (20°C and 10/25°C) than at lower temperatures (Fig. 2b). In general, leachate reduced radicle length to below 5 mm in all temperature regimes, with reduction ranging from 55% at 5/15°C to 74% at 10/25°C.

### Fungal groups and abundance

The distribution of developing fungi in the dishes suggested that these were mostly seed-borne. Application of leachate strongly increased fungal abundance (Fig. 2c;  $F_{1,256} = 6901.5$ ,  $P < 0.0001$ ; explained variance: 88.8%). Compared with the other variables, species effects were small ( $F_{6,256} = 23.3$ ,  $P < 0.0001$ ; explained variance: 1.8%), which implied that fungal development was not, or only weakly, species specific. Fungal growth in the leachate treatment was significantly lower under alternating (88.9  $\pm$  1.2%, mean  $\pm$  SE,  $n = 80$ ) than under constant temperature regimes (96.6  $\pm$  0.6%,  $F_{1,144} = 43.3$ ,  $P < 0.0001$ ; Fig. 2c). The abundance of fungi (Fig. 2c), but not their frequency (Appendix 1), was higher in the leachate treatment than in controls. We classified 14 genera or higher-order groups of fungi (Appendix 1). Six of the genera are known to include pathogenic species, which may produce highly active mycotoxins.

## Discussion

### **Leachate effects on various components of seedling recruitment**

Our laboratory germination experiments showed that leaf leachate of *Stipa pulcherrima*, the dominant species of abandoned dry grasslands in Transylvania, exerted strong negative effects on different processes related to regeneration by seeds of all grassland species studied, and that the strength of the inhibitory effect differed between test species. This is in line with other studies, which demonstrated that leachate from living plant tissues or dead plant remains inhibited seed germination of co-occurring species (e.g. Schlatterer and Tisdale, 1969; Chang-Hung and Chiu-Chung, 1975; Werner, 1975; Bosy and Reader, 1995), but that the degree of susceptibility is species specific. In addition, the leachate effect was prevalent across all constant and fluctuating temperature regimes.

In only two of the species pairs (*Fabaceae* and *Poaceae*) was germination of species with unsuccessful field recruitment significantly more affected by leachate than germination of species with successful field recruitment. The results of our analysis thus led to rejection of our third hypothesis. Our data suggest that chemical effects may only in part be responsible for recruitment failure of the study species under field conditions. Other constraints for successful field recruitment could be very specific requirements for germination (Baskin and Baskin, 2001) or other factors related to the life-cycle of the species.

Timing of germination is a species-specific characteristic determining the success of species under different habitat conditions (Baskin and Baskin, 1988, 2001; Olf et al., 1994; Hölzel and Otte, 2004). As shown by monthly observations of germination in two grassland sites over 2 years (Ruprecht et al., in preparation), field germination of dry grassland species occurred mostly in wet periods during the year, with a highly synchronized germination peak in early spring (March or April). According to our experimental results, leachate application may delay seed germination by about 1.5 weeks on average; germination delay ranged from 0.9 weeks (*S. radiata*) to 2.2 weeks (*G. glaucum*). Due to steep slopes, southern orientation and shallow soils with low water-holding capacity, environmental conditions in these grasslands are especially harsh. With only a relatively narrow time window favourable for germination, seed germination shortly after precipitation events may present a crucial advantage for seedling establishment. Consequently, missing the favourable period for germination and/or slow seedling growth as a consequence of reduced radicle protrusion in the presence of leachate may be fatal for

recruitment from seed and survival of species of this dry grassland system.

Besides specific allelochemical substances, the osmotic potential and pH of litter leachates represent confounding factors that may potentially also inhibit seed germination and seedling development (Wardle et al., 1992). In our study the osmotic potential of the leachates (sterile-filtered as well as unsterilized) was  $-0.16 \pm 0.01$  MPa (mean  $\pm$  SE,  $n = 3$ ), and both had a pH value of  $5.83 \pm 0.01$ . These values certainly differ from the osmotic potential and pH of distilled water, but still lie well within the range where no significant effects on either germination or radicle growth can be expected (Baskin and Baskin, 2001; Black et al., 2006). A preliminary high pressure liquid chromatography (HPLC) analysis of the leaf leachate indicated the presence of coumarin in the *Stipa* leachate. Coumarin is known to have a strong effect on seed germination through irreversible blocking of one or more key physiological events during the early phases of germination (Abenavoli et al., 2006). Additionally, coumarin may affect radicle growth of seedlings (Chon and Kim, 2004), which has also been observed for several other allelochemicals (del Moral and Cates, 1971; Chou and Young, 1975; Norby and Kozlowski, 1980). This effect may also have been observed in the present study. The speed of radicle elongation may be decisive for establishment success, especially in extreme environments where a longer radicle could facilitate easier water and mineral uptake. In this context, the reduction of the radicle to below 5 mm by leachate suggests that *Stipa* leachate may not only influence the germination, but also the establishment of dry grassland species.

### **Were there direct or interactive effects of fungi on germination?**

Besides direct effects on seed germination, allelochemicals may also interact with fungi present on seeds, in the litter or in the soil. These interactions may either be positive (secondary compounds promoting spore germination and the development of hyphae) or negative (toxic effects on fungi or, conversely, decomposing activity of fungi) (Rice, 1984; Rizvi and Rizvi, 1992). In addition, seeds may be the direct target of saprophytic and pathogenic fungi, and fungal-induced seed mortality or delayed germination may have serious implications for plant demography and community processes (Dalling et al., 1998; Blaney and Kotanen, 2001; Mitschunas et al., 2006; Wagner and Mitschunas, 2008).

In our experiment, the development of fungi was apparently promoted by the leachate. Residues of several plant species produce volatile compounds, which may stimulate spore germination and fungal

growth (Menzies and Gilbert, 1967; Rizvi and Rizvi, 1992). Thus, it is likely that some secondary compounds of *S. pulcherrima* may have a similar stimulating effect on the development of seed-borne fungi. Alternatively, coumarin, which was found in the aqueous extracts of *S. pulcherrima* leaves, may delay or prevent the recovery of stable membrane configuration after seed imbibition (Abenavoli *et al.*, 2006). This transient perturbation of membrane structures may cause leakage of solutes from the seeds, which in turn may promote fungal growth.

In light of these complex interactions between plant secondary compounds and fungi, a high abundance of a diverse set of fungi, especially in leachate-treated dishes, presents a confounding factor for the interpretation of results from our first experiment. However, the fact that we obtained identical germination responses after excluding fungi through seed surface and leachate sterilization (at least for the first 3 weeks of germination) supports the view that fungi had little, if any, effects on germination in our experiment. Additionally, leachate effects on percentage germination and seedling radicle elongation were smaller under lower temperatures, which are more likely connected to the way a chemical compound is operating, whereas fungi developed equally well under low and high temperatures, and should thus lead to similar effects across temperature regimes.

### Conclusions

Cessation of traditional management is a serious problem for the conservation of species-rich dry grasslands in Transylvania. Continuous grazing maintains species-rich grasslands with an open structure, dominated by *Stipa lessingiana*, a narrow-leaved feather grass species occurring also in semi-desert plant communities of Asia. After abandonment of grazing, soil erosion induced by grazing animals is slowed down, the number and area of bare patches decreases, and the grassland becomes dominated by *Stipa pulcherrima*, a broad-leaved feather grass with higher biomass production than the former species. The grassland structure is becoming denser, litter is accumulating and, as a long-term consequence, species diversity and evenness is decreasing (Enyedi *et al.*, 2008).

Since the aqueous leaf extract of *S. pulcherrima* inhibited germination and establishment of all study species, it is not unreasonable to suggest that, besides other traits, such as their competitive ability or physical and microclimatic effects of their accumulating litter, chemical litter effects may partly govern the dynamics of dry continental Transylvanian grasslands after abandonment. *S. pulcherrima* itself may evade

autotoxic effects through its perennial life-cycle, which enables it to maintain itself simply through clonal reproduction. Long, hairy awns of its seeds facilitate dispersal over long distances to places where feathergrass abundance and autotoxic effects are low.

However, the fact that germination success observed in the field was not consistently related to the germination responses of species to leaf leachate in our experiments indicates that other factors are equally important for recruitment success in the field. In order to identify these factors and their role for the succession of abandoned dry grasslands, further field and laboratory studies are required.

### Acknowledgements

We thank Christiane Lenz-Kuhl, Joseph Scholz-vom Hofe, Márton Enyedi, Péter L. Pap, Anna Szabó, János Józsa, Mária and Béla Ruprecht for invaluable field and laboratory assistance and Rolf Düring and Uta Kraus for help with the HPLC analysis. We are very grateful to Elke Stein, Karl-Heinz Rexer and Gregor Langen for their help with the determination of fungi and to Hans-Werner Koyro for measuring the osmotic potential of the leachate. Jafargholi Imani is acknowledged for valuable help with the seed sterilization, and Jutta Ahlemeier for statistical advice. Comments of Sandra Burmeier on an earlier version of the manuscript considerably improved this paper. E.R. was supported by a scholarship from the German Academic Exchange Service (DAAD).

### References

- Abenavoli, M.R., Cacco, G., Sorgonà, A., Marabottini, R., Paolacci, A.R., Ciaffi, M. and Badiani, M. (2006) The inhibitory effects of coumarin on the germination of durum wheat (*Triticum turgidum* ssp. *Durum*, CV. Simeto) seeds. *Journal of Chemical Ecology* **32**, 489–506.
- Anaya, A.L. and del Amo, S. (1978) Allelopathic potential of *Ambrosia cumanensis* H.B.K. (Compositae) in a tropical zone of Mexico. *Journal of Chemical Ecology* **4**, 289–304.
- Anderson, R.C., Katz, A.J. and Anderson, M.R. (1978) Allelopathy as a factor in the success of *Helianthus mollis* Lam. *Journal of Chemical Ecology* **4**, 9–16.
- Bakker, J.P. and Berendse, F. (1999) Constraints in the restoration of ecological diversity in grassland and heathland communities. *Trends in Ecology and Evolution* **14**, 63–68.
- Baskin, C.C. and Baskin, J.M. (1988) Germination ecophysiology of herbaceous plant species in a temperate region. *American Journal of Botany* **75**, 286–305.
- Baskin, C.C. and Baskin, J.M. (2001) *Seeds. Ecology, biogeography, and evolution of dormancy and germination*. San Diego, Academic Press.
- Black, M., Bewley, J.D. and Halmer, P. (2006) *The encyclopedia of seeds – science, technology and uses*. Wallingford, CABI Publishing.



- Blaney, C.S. and Kotanen, P.M.** (2001) Effects of fungal pathogens on seeds of native and exotic plants: a test using congeneric pairs. *Journal of Applied Ecology* **38**, 1104–1113.
- Bosy, J.L. and Reader, R.J.** (1995) Mechanisms underlying the suppression of forb seedlings by grass (*Poa pratensis*) litter. *Functional Ecology* **9**, 635–639.
- Chang-Hung, C. and Chiu-Chung, Y.** (1975) Phytotoxic substances in twelve subtropical grasses. *Journal of Chemical Ecology* **1**, 183–193.
- Chon, S.U. and Kim, Y.M.** (2004) Herbicidal potential and quantification of suspected allelochemicals from four grass crop extracts. *Journal of Agronomy and Crop Science* **190**, 145–150.
- Chou, C.H. and Young, C.C.** (1975) Phytotoxic substances in twelve subtropical grasses. *Journal of Chemical Ecology* **1**, 183–193.
- Dalling, J.W., Swaine, M.D. and Garwood, N.C.** (1998) Dispersal patterns and seed bank dynamics of pioneer trees in moist tropical forests. *Ecology* **79**, 564–578.
- Datta, S.C. and Sinha-Roy, S.P.** (1975) Phytotoxic effects of *Croton bonplandianum* Baill. on weedy associates. *Vegetatio* **30**, 157–163.
- del Moral, R. and Cates, R.G.** (1971) Allelopathic potential of the dominant vegetation of Western Washington. *Ecology* **52**, 1030–1037.
- Donath, T.W. and Eckstein, R.L.** (2008) Grass and oak litter exert different effects on seedling emergence of herbaceous perennials from grasslands and woodlands. *Journal of Ecology* **96**, 272–280.
- Eckstein, R.L. and Donath, T.W.** (2005) Interactions between litter and water availability affect seedling emergence in four familial pairs of floodplain species. *Journal of Ecology* **93**, 807–816.
- Ellenberg, E.** (1988) *Vegetation ecology of Central Europe*. Cambridge, Cambridge University Press.
- Enyedi, Z.M., Ruprecht, E. and Deák, M.** (2008) Long-term effects of the abandonment of grazing on steppe-like grasslands. *Applied Vegetation Science* **11**, 53–60.
- Facelli, J.M. and Pickett, S.T.A.** (1991) Plant litter: its dynamics and effects on plant community structure. *Botanical Review* **57**, 1–31.
- Grime, J.P., Cornelissen, J.H.C., Thompson, K. and Hodgson, J.G.** (1996) Evidence of a causal connection between anti-herbivore defence and the decomposition rate of leaves. *Oikos* **77**, 489–494.
- Harborne, J.B.** (1993) *Introduction to ecological biochemistry*. New York, Academic Press.
- Hölzel, N. and Otte, A.** (2004) Ecological significance of seed germination characteristics in flood-meadow species. *Flora* **199**, 12–24.
- Jensen, K. and Gutekunst, K.** (2003) Effects of litter on establishment of grassland plant species: the role of seed size and successional status. *Basic and Applied Ecology* **4**, 579–587.
- Kahmen, S., Poschlod, P. and Schreiber, K.F.** (2002) Conservation management of calcareous grasslands. Changes in plant species composition and response of functional traits during 25 years. *Biological Conservation* **104**, 319–328.
- Korneck, D., Schnittler, M., Klingenstein, F., Ludwig, G., Takla, M., Bohn, U. and May, R.** (1998) Warum verarmt unsere Flora? Auswertung der Roten Listen der Farn- und Blütenpflanzen Deutschlands. *Schriftenreihe für Vegetationskunde* **29**, 299–444.
- Luoto, M., Pykälä, J. and Kuussaari, M.** (2003) Decline of landscape-scale habitat and species diversity after the end of cattle grazing. *Journal for Nature Conservation* **11**, 171–178.
- Mazzoleni, S., Bonanomi, G., Giannino, F., Rietkerk, M., Dekker, S.C. and Zucconi, F.** (2007) Is plant biodiversity driven by decomposition processes? An emerging new theory on plant diversity. *Community Ecology* **8**, 103–109.
- Menzies, J.D. and Gilbert, R.G.** (1967) Response of soil microflora to soil volatile components in plant residues. *Soil Science Society of America Journal* **31**, 495–496.
- Mitchley, J. and Xofis, P.** (2005) Landscape structure and management regime as indicators of calcareous grassland habitat condition and species diversity. *Journal for Nature Conservation* **13**, 171–183.
- Mitschunas, N., Wagner, M. and Filser, J.** (2006) Evidence for a positive influence of fungivorous soil invertebrates on the seed bank persistence of grassland species. *Journal of Ecology* **94**, 791–800.
- Moog, D., Poschlod, P., Kahmen, S. and Schreiber, K.F.** (2002) Comparison of species composition between different grassland management treatments after 25 years. *Applied Vegetation Science* **5**, 99–106.
- Myster, R.W.** (2006) Species-specific effects of grass litter mass and type on emergence of three tall grass prairie species. *Écoscience* **13**, 95–99.
- Newman, E.I. and Rovira, A.D.** (1975) Allelopathy among some British grassland species. *Journal of Ecology* **63**, 727–737.
- Norby, R.J. and Kozlowski, T.T.** (1980) Allelopathic potential of ground cover species on *Pinus resinosa* seedlings. *Plant and Soil* **57**, 363–374.
- Olf, H., Pegtel, D.M., van Groenendaal, J.M. and Bakker, J.P.** (1994) Germination strategies during grassland succession. *Journal of Ecology* **82**, 69–77.
- Pärtel, M., Zobel, M., Zobel, K. and van der Maarel, E.** (1996) The species pool and its relation to species richness: evidence from Estonian plant communities. *Oikos* **75**, 111–117.
- Pykälä, J., Luoto, M., Heikkinen, R.K. and Kontula, T.** (2005) Plant species richness and persistence of rare plants in abandoned semi-natural grasslands in northern Europe. *Basic and Applied Ecology* **6**, 25–33.
- Quested, H. and Eriksson, O.** (2006) Litter species composition influences the performance of seedlings of grassland herbs. *Functional Ecology* **20**, 522–532.
- Quested, H., Eriksson, O., Fortunel, C. and Garnier, E.** (2007) Plant traits relate to whole-community litter quality and decomposition following land use change. *Functional Ecology* **21**, 1016–1026.
- Quinn, G.P. and Keough, M.J.** (2002) *Experimental design and data analysis for biologists*. Cambridge, Cambridge University Press.
- Rice, E.L.** (1972) Allelopathic effects of *Andropogon virginicus* and its persistence in old fields. *American Journal of Botany* **59**, 752–755.
- Rice, E.L.** (1984) *Allelopathy* (2nd edition). San Diego, Academic Press.

- Rizvi, S.J.H. and Rizvi, V.** (1992) *Allelopathy. Basic and applied aspects*. London, Chapman & Hall.
- Schlatterer, E.F. and Tisdale, E.W.** (1969) Effects of litter of *Artemisia*, *Chrysothamnus*, and *Tortula* on germination and growth of three perennial grasses. *Ecology* **50**, 869–873.
- Tilman, D.** (1993) Species richness of experimental productivity gradients: how important is colonisation limitation? *Ecology* **74**, 2179–2191.
- Tutin, T.G., Heywood, V.H., Burges, N.A., Moore, D.M., Valentine, D.H., Walters, S.M. and Webb, D.A.** (1964–1980) *Flora Europaea*. Vols 1–5. Cambridge, Cambridge University Press.
- Vandvik, V. and Birks, H.J.B.** (2002) Partitioning floristic variance in Norwegian upland grasslands into within-site and between-site components: are the patterns determined by environment or by land-use? *Plant Ecology* **162**, 233–245.
- Virágh, K. and Bartha, S.** (1996) The effect of current dynamical state of a loess steppe community on its responses to disturbances. *Tiscia* **30**, 3–13.
- Wagner, M. and Mitschunas, N.** (2008) Fungal effects on seed bank persistence and potential applications in weed biocontrol: a review. *Basic and Applied Ecology* **9**, 191–203.
- Waldhardt, R. and Otte, A.** (2003) Indicators of plant species and community diversity in grasslands. *Agriculture, Ecosystems and Environment* **98**, 339–351.
- Wardle, D.A., Nicholson, K.S. and Ahmed, M.** (1992) Comparison of osmotic and allelopathic effects of grass leaf extracts on grass seed germination and radicle elongation. *Plant and Soil* **140**, 315–319.
- Wardle, D.A., Nicholson, K.S. and Rahman, A.** (1993) Influence of plant age on the allelopathic potential of nodding thistle (*Carduus nutans* L.) against pasture grasses and legumes. *Weed Research* **33**, 69–78.
- Wardle, D.A., Nilsson, M.C., Gallet, C. and Zackrisson, O.** (1998) An ecosystem-level perspective of allelopathy. *Biological Reviews* **73**, 305–319.
- Weier, E. and Keddy, P.** (1999) *Ecological assembly rules: Perspectives, advances, retreats*. Cambridge, Cambridge University Press.
- Wellstein, C., Otte, A. and Waldhardt, R.** (2007) Impact of site and management on the diversity of Central European mesic grassland. *Agriculture, Ecosystems and Environment* **122**, 203–210.
- Werner, P.A.** (1975) The effects of plant litter on germination in teasel, *Dipsacus sylvestris* Huds. *American Midland Naturalist* **94**, 470–476.

## Appendix

The frequency of fungi (genera or higher-order groups) in samples treated with distilled water or leachate under different temperature regimes

Temperature (°C)	10		5/15		20		10/25		
	Water	Leachate	Water	Leachate	Water	Leachate	Water	Leachate	
Treatment	Sapr/Path								
Fungal genera (groups)	Sapr/Path								
<i>Mucorales</i> ( <i>Rhizopus</i> , <i>Mucor</i> )	Sapr	–	40	–	40	15	40	–	40
<i>Alternaria</i>	>>Path*	35	38	39	40	36	35	39	38
<i>Botrytis</i>	Path	38	34	12	13	24	15	39	27
<i>Fusarium</i>	>>Path*	10	25	9	21	9	28	2	27
<i>Epicoccum</i>	Sapr	14	–	4	1	–	–	9	10
<i>Aureobasidium</i>	Sapr	2	–	16	6	–	–	–	–
<i>Sordaria</i>	Sapr	–	3	14	3	–	–	1	8
<i>Rhizoctonia</i>	Path	–	–	3	5	7	7	–	1
<i>Aspergillus</i>	Sapr	–	–	3	–	–	2	1	–
<i>Myxomycetes</i>	Sapr	–	1	–	–	1	–	1	–
<i>Melanospora</i>	Mycoparasite	3	5	–	–	3	–	–	–
<i>cf. Drechslera</i>	>>Path	–	–	–	–	1	–	–	–
<i>Chaetomium</i>	Sapr	–	–	–	–	–	1	1	1
<i>Helminthosporium</i>	Path*	–	–	–	–	–	–	–	1
ND (not determined)		–	–	–	–	–	–	–	12

Fungal groups may be saprophytic (Sapr) or pathogenic (Path) (>>, mainly pathogenic); some groups are known to produce toxic substances (\*).