

## Acid and Temperature Treatments Result in Increased Germination of Seeds of Three Fescue Species

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### Abstract

Efficient germination of fescue seeds is essential for successful establishment of meadows and pastures. This research was conducted to ascertain the effects of various acid and temperature treatments on seed germination in three fescue species: *Festuca rubra*, *F. ovina*, and *F. pratensis*. Seeds from different cultivars, populations, or lots were exposed either to four concentrations of sulfuric acid at three different time intervals (12 treatments) or six different temperatures at three different time intervals (18 treatments). Despite all belonging to the genus *Festuca*, the seed from different species responded differently to the treatments. The three optimum treatments for *F. rubra* seed involved soaking in a 75% solution of sulfuric acid for 20 minutes (improved the germination rate by 19%), soaking in a 50% solution of sulfuric acid for 30 minutes (improved the germination rate by 18%) and exposure to either 60°C or 70°C for 90 minutes (improved the germination rate by 17%). For *F. ovina* seed, optimal treatments included soaking seeds for either 10 or 20 minutes in a 50% sulfuric acid solution (both treatments improved germination rates by 13%) or exposing seeds for 30 minutes in a 25% sulfuric acid solution and 80°C for 60 minutes (improved germination rate by 12%). Two optimal treatments were identified for *F. pratensis* seed. Whereas the first involved soaking the seeds in a 75% sulfuric acid solution for 30 minutes (improved germination rates by 22%), the second involved either exposing the seeds to 90°C for 90 or 60 minutes, or exposing the seeds to 80°C for 90 minutes (improved germination rate by 21%). Our findings indicate that if fescue seed is to be sown during the autumn (two to three months after seed collecting), treating it with acid and temperature can significantly enhance its germination.

**Keywords:** acid treatments, fescue, seed germination, temperature treatments

### Introduction

Red fescue (*Festuca rubra*), sheep fescue (*F. ovina*), and meadow fescue (*F. pratensis*) are the subjects of intensive research. All are valued for both forage production and special purposes, ranging from the preparation of sport grounds, parks, and house gardens, to preventing the erosion of soil from embankments and railroads.

When grown under the agroecological conditions found in Serbia, biomass accumulation of red fescue can reach approximately 7-10 t ha<sup>-1</sup> of dry matter per year; however, it is more commonly grown in grass-legume mixtures, resulting in substantially higher forage yield and quality (Tomić *et al.*, 2007). Red fescue, especially populations taken from natural environments, makes a good quality component for turf grass (Kroumov, 2009) and slope grass used to cover ski runs to prevent soil erosion (Bjedov *et al.*, 2011). It is also beneficial for the recultivation of ash deposits (Mitrović *et al.*, 2008).

Red fescue and sheep fescue have been extensively studied and show promising results with regard to preparing sport grounds (Dernoeden, 1998) and decorating parks

and home-gardens (Casler, 2006). Both species have been studied for use in parks under Mediterranean conditions, although the use of neither was as attractive as the use of tall fescue (*F. arundinacea*) (Demiroğlu *et al.*, 2010). Decorative applications of red fescue have not been extensively studied in continental Southeastern Europe. Red fescue is a dominant species in the mountainous region of Stara planina in Eastern Serbia and Western Bulgaria. Although sheep fescue is less established, both species are important for sheep and goat grazing (Tomić *et al.*, 2011).

Meadow fescue (*F. pratensis*) is a classic forage species, which produces high outputs of forage dry matter (approximately 10 t ha<sup>-1</sup> per year). It is grown primarily in grass-legume mixtures, resulting in considerably higher yield and better forage quality (Tomić *et al.*, 2007). Meadow fescue noticeably improves grassland forage quality under natural meadow conditions (Tomić *et al.*, 2010; Vintu *et al.*, 2011).

Under natural conditions, seed dormancy and postponed germination of forage grasses may increase the overall percentage of seeds that eventually germinate, and promote seedling emergence during frost-free periods

(Simpson, 1990; Stanisavljević *et al.*, 2010c). However, successfully growing these species requires a high and consistent germination rate, coupled with fast and vigorous seedling growth. On the other hand, there are dormant grass seed that never germinate, regardless of whether conditions are favorable (Simpson, 1990). The complex physiological and biochemical processes in seeds that dictate the level of seed dormancy and the inability of seeds to germinate (Bewley, 1997) vary according to the genetic origin of the seeds and its environment during growth, even including the position of the seed on the parent plant (Andersson and Milberg, 1998; Fenner, 1991).

The aim of the experiment was to determine the level of seed dormancy and germination of seeds of fescue species, both immediately upon harvesting and two and a half months after harvesting, as well as to determine an optimum acid and temperature treatment for breaking dormancy and enhancing the germination rate.

### Materials and methods

The fescue seeds were taken from near the city of Kruševac (Central Serbia) and the mountainous regions of Eastern and Southern Serbia. Batches of seed of meadow fescue cv. 'K-21', originally derived from commercial seed production, were harvested at three locations, and the seed was cleaned manually (Tab. 1). Cultivars, populations, and lots were tested to evaluate the impact of treatments with 98%, 75%, 50%, and 25% solutions of sulfuric acid. Aliquots of the seed were each soaked in one of the four different concentrations of acid for either 30, 20, or 10 minutes. After soaking, the seed was rinsed with distilled water for 10 minutes. The effects of different tem-

peratures (90°C, 80°C, 70°C, 60°C, 50°C, and 40°C) on fescue seeds were also investigated. Seeds were exposed to each of these temperatures for 90, 60, and 30 minutes before their rates of germination were tested.

### Germination test

Germination rate and seed dormancy without any acid or temperature treatments were established immediately after seeds had been collected and dried. After storage for two and a half months, acid and temperature treatments were applied to 8 batches of seeds, each containing 100 seeds. During germination, seeds were incubated at 25°C for eight hours in light and at 15°C for 16 hours in darkness. Seeds were germinated on filter paper moistened with water. For meadow and red fescue, germinated seedlings were counted 14 days after seeds were sown, whereas for sheep fescue germinated seedlings were counted 21 days after seeds were sown. The tetrazolium test for seed viability, which was performed in accordance with ISTA rules (ISTA, 2011), was used to establish whether ungerminated seeds were either dormant or dead.

### Statistical analysis

The data were analysed using parametric tests (ANOVA and LSD test) and the Statistica 8.0 software package (StatSoft, Inc.). For statistical analysis, germination and dormancy-related data percentages were arcsine transformed.

### Results and discussion

Significant differences in both germination rate and dormancy ( $p \leq 0.05$ ) were detected between seeds of sheep

Tab. 1. Cultivars, populations, lots, regions, and locations from where seed was sourced

Red fescue		Sheep fescue		Meadow fescue	
Seed category and seed source	Site	Seed category and seed source	Site	Seed category and seed source	Site
Buki (fp and sp)	44° 48' N	Ridu (sp)	43° 34' N	'K-21' Lot	43° 50' N
Northern Bosnia and Herzegovina and (1)	17° 13' E 145 m a.s.l.	Central Serbia (1)	21° 34' E 575 m a.s.l.	Eastern Serbia (1)	22° 22' E 351 m a.s.l.
Rtanj (p)	43° 48' N	Deli Jovan I (p)	44° 10' N	'K-21' Lot	43° 53' N
Eastern Serbia (2)	21° 41' E 364 m a.s.l.	Eastern Serbia (2)	22° 16' E 445 m a.s.l.	Eastern Serbia (2)	22° 18' E 128 m a.s.l.
Čestobrodica (p)	43° 50' N	Rtanj I (p)	43° 46' N	'K-21' Lot	43° 17' N
Eastern Serbia (3)	21° 39' E 570 m a.s.l.	Eastern Serbia (3)	21° 56' E 763 m a.s.l.	Southern Serbia (3)	21° 56' E 265 m a.s.l.
Tresibaba (p)	43° 51' N	Rtanj II (p)	43° 43' N	'K-21' Lot	44° 48' N
Southern Serbia (4)	22° 76' E 419 m a.s.l.	Eastern Serbia (4)	21° 56' E 733 m a.s.l.	Central Serbia (4)	17° 13' E 145 m a.s.l.
Maxima (sp)	44° 48' N	Tupišnica I (p)	43° 50' N	'K-21' Lot	43° 24' N
Central Serbia (5)	17° 13' E 145 m a.s.l.	Eastern Serbia (5)	21° 41' E 560 m a.s.l.	Southern Serbia (5)	21° 48' E 206 m a.s.l.
'K-14' (fp)	44° 48' N	Tupišnica II (p)	43° 50' N	'K-21' Lot	43° 34' N
Central Serbia (6)	17° 13' E 145 m a.s.l.	Eastern Serbia (6)	21° 41' E 560 m a.s.l.	Central Serbia (6)	21° 19' E 232 m a.s.l.

a.s.l., above sea-level; fp, cultivar is intended for forage production; sp, special purpose variety; p, population

Tab. 2. Germination rate (G, %) and dormancy (D, %) of fescue species seeds, immediately upon harvesting

Fescue species		Cultivar - population - lot						$\bar{X}$	CV %
		1	2	3	4	5	6		
Red fescue	G	66	62	67	64	60	67	64 <sup>AB</sup>	4.5
	D	30	33	31	31	34	31	32 <sup>ab</sup>	4.8
Sheep fescue	G	72	68	71	70	68	70	70 <sup>A</sup>	2.3
	D	30	31	28	27	31	29	29 <sup>b</sup>	5.6
Meadow fescue	G	62	59	61	63	60	62	61 <sup>B</sup>	2.4
	D	35	33	34	34	32	32	33 <sup>a</sup>	3.6

Different letters between cultivars denote significant differences (LSD test,  $p < 0.05$ )

and meadow fescue immediately after they were collected and dried (Tab. 2). Germination of seeds from different cultivars and populations of red fescue had a variation coefficient of 4.5%. Smaller variation coefficients for germination were determined in seed of varieties and populations of sheep fescue (CV = 2.3%) and meadow fescue (CV = 2.4%).

In the continental region of Central and Southeastern Europe, approximately two and a half months elapses between the time of seed collection and the sowing of forage or amenity grasses during the autumn. Sowing in this period provides sufficient time for germination, seedling development, and survival during the winter. Compared with sowing seed during the spring of the following year, autumn sowing provides for substantially better turf formation, more forage yield, and increased seed yield (Stanisavljević et al., 2010c). Amenity grasses and sport turfgrasses can be intensively managed using optimal conditions for soil-bed preparation, irrigation and fertilization, with agroecological conditions playing little part. Under these circumstances, sowing during the autumn is

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Tab. 3. The influence of acid and temperature treatments on the germination rate and dormancy of seeds from different varieties and populations of red fescue

Treatment		Cultivar or population														CV %	
		Germination %						CV %	Dormant seeds %						CV %		
		1	2	3	4	5	6		$\bar{X}$	1	2	3	4	5			6
Control		73	69	69	72	70	71	71 <sup>g</sup>	2.3	25	24	26	25	24	27	25 <sup>a</sup>	4.7
H <sub>2</sub> SO <sub>4</sub> 98%	30'	0	0	0	0	0	0	0 <sup>h</sup>	-	0	0	0	0	0	0	0 <sup>i</sup>	-
	20'	0	0	0	0	0	0	0 <sup>h</sup>	-	0	0	0	0	0	0	0 <sup>i</sup>	-
	10'	1	1	0	1	0	0	1 <sup>h</sup>	110	0	0	0	0	0	0	0 <sup>i</sup>	-
H <sub>2</sub> SO <sub>4</sub> 75%	30'	74	70	76	71	82	79	75 <sup>e-g</sup>	6.2	0	0	0	0	0	0	0 <sup>i</sup>	-
	20'	89	85	95	85	96	92	90 <sup>a</sup>	5.3	0	0	0	0	0	0	0 <sup>i</sup>	-
	10'	90	80	87	87	89	89	87 <sup>a-d</sup>	4.2	0	0	0	0	0	0	0 <sup>i</sup>	-
H <sub>2</sub> SO <sub>4</sub> 50%	30'	88	84	92	86	94	87	89 <sup>a-b</sup>	4.3	0	0	0	0	0	0	0 <sup>i</sup>	-
	20'	86	83	88	84	92	89	87 <sup>a-d</sup>	3.9	1	0	1	2	1	0	1 <sup>i</sup>	90.3
	10'	83	80	87	81	90	88	85 <sup>a-c</sup>	4.8	3	2	3	4	3	2	3 <sup>h-i</sup>	26.6
H <sub>2</sub> SO <sub>4</sub> 25%	30'	86	77	86	83	87	76	83 <sup>a-f</sup>	5.9	6	5	7	6	5	4	6 <sup>h</sup>	19.1
	20'	84	74	80	80	83	75	79 <sup>a-g</sup>	5.2	7	8	8	6	7	6	7 <sup>e-h</sup>	12.8
	10'	80	72	76	79	77	73	76 <sup>d-g</sup>	4.2	9	8	9	8	9	7	8 <sup>e-g</sup>	9.8
T 90°C	90'	77	75	72	71	73	79	75 <sup>e-g</sup>	4.1	0	0	0	0	0	0	0 <sup>i</sup>	-
	60'	80	78	77	79	78	80	79 <sup>a-g</sup>	1.5	0	0	0	0	0	0	0 <sup>i</sup>	-
	30'	82	75	76	77	76	79	78 <sup>b-g</sup>	3.3	0	0	0	0	0	0	0 <sup>i</sup>	-
T 80°C	90'	79	78	77	80	79	82	79 <sup>a-g</sup>	2.2	0	0	0	0	0	0	0 <sup>i</sup>	-
	60'	83	80	80	81	78	80	80 <sup>a-g</sup>	2.0	4	3	5	3	4	5	4 <sup>g-i</sup>	22.4
	30'	85	82	79	86	76	78	81 <sup>a-g</sup>	4.9	6	5	7	4	5	6	6 <sup>h</sup>	19.1
T 70°C	90'	90	89	89	88	87	86	88 <sup>a-c</sup>	1.7	2	3	1	4	2	3	3 <sup>h-i</sup>	42.0
	60'	91	90	88	88	76	78	85 <sup>a-c</sup>	7.6	5	6	5	7	6	7	6 <sup>h</sup>	14.9
	30'	88	86	76	84	85	76	83 <sup>a-f</sup>	6.3	8	7	6	8	8	8	8 <sup>e-g</sup>	11.2
T 60°C	90'	89	88	88	87	86	88	88 <sup>a-c</sup>	1.2	5	7	8	7	5	7	7 <sup>e-h</sup>	18.8
	60'	86	84	83	82	84	83	84 <sup>a-c</sup>	1.6	9	8	9	9	8	9	9 <sup>e-f</sup>	6.0
	30'	83	80	81	79	81	81	81 <sup>a-g</sup>	1.6	10	9	11	12	11	12	11 <sup>d-e</sup>	10.8
T 50°C	90'	83	81	79	80	79	78	80 <sup>a-g</sup>	2.2	6	8	9	10	8	9	8 <sup>e-g</sup>	16.4
	60'	80	79	76	77	76	74	77 <sup>c-g</sup>	2.9	10	12	11	12	11	12	11 <sup>d-e</sup>	7.2
	30'	78	76	75	76	74	73	75 <sup>e-g</sup>	2.3	13	14	15	14	15	13	14 <sup>d</sup>	6.4
T 40°C	90'	81	79	76	77	76	74	77 <sup>c-g</sup>	3.2	10	15	19	18	20	19	17 <sup>c</sup>	22.4
	60'	78	75	73	73	74	73	74 <sup>e-g</sup>	2.6	14	20	21	22	20	20	20 <sup>b</sup>	14.4
	30'	75	72	70	71	72	72	72 <sup>f-g</sup>	2.3	22	23	23	23	23	25	23 <sup>a</sup>	4.2

Different letters between cultivars denote significant differences (LSD test,  $p < 0.05$ )

Tab. 4. The influence of acid and temperature treatments on the germination rate and dormancy of seed from various sheep fescue populations and varieties

Treatment	Cultivar or population																
	Germination %							CV %	Dormant seeds %						CV %		
	1	2	3	4	5	6	$\bar{X}$		1	2	3	4	5	6		$\bar{X}$	
Control	72	68	71	70	68	69	70 <sup>b</sup>	2.3	23	24	22	26	22	24	24 <sup>a</sup>	6.5	
H <sub>2</sub> SO <sub>4</sub> 98%	30'	0	0	0	0	0	0 <sup>e</sup>	-	0	0	0	0	0	0	0 <sup>h</sup>	-	
	20'	0	0	0	0	0	0 <sup>e</sup>	-	0	0	0	0	0	0	0 <sup>h</sup>	-	
	10'	0	0	0	0	0	0 <sup>e</sup>	-	0	0	0	0	0	0	0 <sup>h</sup>	-	
H <sub>2</sub> SO <sub>4</sub> 75%	30'	48	45	41	40	42	42 <sup>d</sup>	10.6	0	0	0	0	0	0	0 <sup>h</sup>	-	
	20'	51	49	48	44	46	47 <sup>d</sup>	5.3	0	0	0	0	0	0	0 <sup>h</sup>	-	
	10'	64	53	57	51	60	57 <sup>c</sup>	8.3	0	0	0	0	0	0	0 <sup>h</sup>	-	
H <sub>2</sub> SO <sub>4</sub> 50%	30'	76	72	70	66	70	71 <sup>ab</sup>	4.7	0	0	0	0	0	0	0 <sup>h</sup>	-	
	20'	90	86	80	79	86	83 <sup>a</sup>	6.7	0	0	0	0	0	0	0 <sup>h</sup>	-	
	10'	88	84	80	76	85	83 <sup>a</sup>	5.1	0	0	0	0	0	0	0 <sup>h</sup>	-	
H <sub>2</sub> SO <sub>4</sub> 25%	30'	88	82	79	78	85	82 <sup>a</sup>	4.6	4	2	3	2	4	4	3 <sup>fh</sup>	31.1	
	20'	85	79	75	73	74	77 <sup>ab</sup>	5.8	5	4	4	3	6	6	5 <sup>fh</sup>	26.0	
	10'	82	75	73	71	68	73 <sup>ab</sup>	6.8	7	6	5	4	7	7	6 <sup>eg</sup>	21.1	
T 90°C	90'	75	73	74	73	77	75 <sup>ab</sup>	2.2	0	0	0	0	0	0	0 <sup>h</sup>	-	
	60'	78	74	76	76	79	77 <sup>ab</sup>	2.6	0	0	0	0	0	0	0 <sup>h</sup>	-	
	30'	79	77	79	76	81	82	79 <sup>ab</sup>	2.9	0	0	0	0	0	0 <sup>h</sup>	-	
T 80°C	90'	80	75	77	82	83	80 <sup>ab</sup>	4.2	0	0	0	0	0	0	0 <sup>h</sup>	-	
	60'	83	80	79	84	82	84	82 <sup>a</sup>	2.6	0	1	0	2	0	1	1 <sup>g-h</sup>	122.1
	30'	80	77	77	81	81	81	80 <sup>ab</sup>	2.5	2	3	4	3	1	3	3 <sup>fh</sup>	38.7
T 70°C	90'	81	80	79	78	83	84	81 <sup>ab</sup>	2.9	0	0	0	0	0	0	0 <sup>h</sup>	-
	60'	78	76	77	76	77	78	77 <sup>ab</sup>	1.2	3	3	5	4	5	5	4 <sup>fh</sup>	23.6
	30'	76	74	75	76	75	76	75 <sup>ab</sup>	1.1	5	7	6	7	6	4	6 <sup>eg</sup>	20.0
T 60°C	90'	81	81	80	82	83	81 <sup>ab</sup>	1.4	3	4	5	3	5	4	4 <sup>fh</sup>	22.4	
	60'	76	74	74	74	75	75 <sup>ab</sup>	1.7	6	7	7	6	8	7	7 <sup>ef</sup>	11.0	
	30'	75	73	73	73	74	74	74 <sup>ab</sup>	1.1	10	9	10	11	9	8	10 <sup>de</sup>	11.0
T 50°C	90'	80	77	78	79	81	79	79 <sup>ab</sup>	1.8	6	6	8	6	9	6	7 <sup>ef</sup>	19.5
	60'	75	73	74	73	73	73	74 <sup>ab</sup>	1.1	9	10	9	8	10	12	10 <sup>de</sup>	14.1
	30'	74	73	73	72	73	73	73 <sup>ab</sup>	0.9	12	14	11	13	14	9	12 <sup>d</sup>	16.0
T 40°C	90'	78	76	77	77	79	77 <sup>ab</sup>	1.3	8	14	17	15	14	16	14 <sup>cd</sup>	22.6	
	60'	74	72	72	74	72	75	73 <sup>ab</sup>	1.8	9	18	19	18	17	19	17 <sup>bc</sup>	23.0
	30'	72	70	72	74	70	71 <sup>ab</sup>	2.3	20	21	20	19	20	21	20 <sup>b</sup>	3.7	

Different letters between cultivars denote significant differences (LSD test,  $p < 0.05$ )

superior to sowing during the spring (Salehi and Khosh-Khui, 2004).

On the other hand, current seed trading regulations in the Southeastern European countries require a germination rate that exceeds 70%. Currently the germination rate of tall oatgrass (*Arrhenatherum elatius*) is 63% (Stanisavljević et al., 2010a), that of Italian ryegrass (*Lolium multiflorum*) is 66%, that of cocksfoot (*Dactylis glomerata*) is 57% (Stanisavljević et al., 2011), and that of meadow fescue (*F. pratensis*) is approximately 63% (Stanisavljević et al., 2010b). These germination rates prevent commercial sale of the seed in southeastern Europe. Red fescue, tall fescue, and meadow fescue all belong to the genus *Festuca*, and all reach a germination rate of 75% after a seed-ripening period that lasts two and a half months (Stanisavljević et al., 2010b). In contrast with the situation for seeds of *Festuca*

species, Rozman et al. (2010) reported different seed germination rates among seeds of different *Lolium* species.

#### Red fescue

Sulfuric acid and different temperatures had different effects on the breaking of dormancy and enhancement of germination rates of *F. rubra* seeds. Ripening over the course of two and a half months reduced dormancy of red fescue seed by 7% and increased the germination rate by 7%, when data from all *F. rubra* genotypes were averaged (Tab. 3). Soaking seeds for 20 minutes in a 75% solution of sulfuric acid proved to be the treatment most effective with regard to increasing germination rates, promoting a 19% increase in germination compared with the control treatment. The next most effective treatment involved soaking the seeds in a 50% sulphuric acid solution for 30

Tab. 5. The influence of acid and temperature treatments on the germination rate and dormancy of seed from different lots of meadow fescue seed

Treatment	Seed lots																
	Germination %							CV %	Dormant seeds %						CV %		
	1	2	3	4	5	6	$\bar{X}$		1	2	3	4	5	6		$\bar{X}$	
Control	70	69	72	72	71	71	71 <sup>f</sup>	1.7	25	27	26	24	28	26	26 <sup>a</sup>	5.4	
H <sub>2</sub> SO <sub>4</sub> 98%	30'	0	0	0	0	0	0 <sup>g</sup>	-	0	0	0	0	0	0	0 <sup>j</sup>	-	
	20'	2	4	3	2	3	4	3 <sup>g</sup>	29.8	0	0	0	0	0	0 <sup>j</sup>	-	
	10'	6	7	10	9	8	9	8 <sup>g</sup>	18.0	0	0	0	0	0	0 <sup>j</sup>	-	
H <sub>2</sub> SO <sub>4</sub> 75%	30'	92	94	95	91	93	91	93 <sup>a</sup>	1.8	0	0	0	0	0	0 <sup>j</sup>	-	
	20'	89	91	91	90	89	88	90 <sup>a-c</sup>	1.4	0	0	0	0	0	0 <sup>j</sup>	-	
	10'	88	85	87	86	85	83	86 <sup>a-c</sup>	2.0	0	0	0	0	0	0 <sup>j</sup>	-	
H <sub>2</sub> SO <sub>4</sub> 50%	30'	89	90	92	89	88	90	90 <sup>a-c</sup>	1.5	0	0	0	0	0	0 <sup>j</sup>	-	
	20'	85	82	86	83	82	81	83 <sup>a-c</sup>	2.3	0	0	0	0	0	0 <sup>j</sup>	-	
	10'	82	81	83	81	80	80	81 <sup>a-f</sup>	1.4	1	1	1	1	0	0	1 <sup>j</sup>	77.5
H <sub>2</sub> SO <sub>4</sub> 25%	30'	83	82	84	82	83	79	82 <sup>a-c</sup>	2.1	0	0	0	0	0	0 <sup>j</sup>	-	
	20'	81	80	82	81	83	78	81 <sup>a-f</sup>	2.1	1	2	1	1	1	1 <sup>j</sup>	35.0	
	10'	80	78	80	79	80	76	79 <sup>c-f</sup>	2.0	4	3	2	4	2	3	3 <sup>j</sup>	29.8
T 90°C	90'	91	93	93	91	92	89	92 <sup>a-b</sup>	1.7	0	0	0	0	0	0 <sup>j</sup>	-	
	60'	89	94	94	89	95	92	92 <sup>a-b</sup>	2.9	0	0	0	0	0	0 <sup>j</sup>	-	
	30'	89	89	91	88	93	88	90 <sup>a-c</sup>	2.2	0	0	0	0	0	0 <sup>j</sup>	-	
T 80°C	90'	90	92	95	90	94	89	92 <sup>a-b</sup>	2.6	0	0	0	0	0	0 <sup>j</sup>	-	
	60'	87	89	93	87	92	87	89 <sup>a-d</sup>	3.0	1	0	1	1	1	0	1 <sup>j</sup>	77.5
	30'	88	88	92	86	91	95	90 <sup>a-c</sup>	3.7	4	4	3	4	3	3	4 <sup>h-j</sup>	15.7
T 70°C	90'	89	91	93	88	91	88	90 <sup>a-c</sup>	2.2	0	0	0	0	0	0 <sup>j</sup>	-	
	60'	87	89	90	86	90	87	88 <sup>a-d</sup>	2.0	5	4	5	4	5	5	5 <sup>h-i</sup>	11.1
	30'	86	87	88	85	89	85	87 <sup>a-d</sup>	1.9	7	8	6	8	6	7	7 <sup>g-h</sup>	12.8
T 60°C	90'	88	87	89	86	87	86	87 <sup>a-d</sup>	1.3	1	2	1	2	1	1	1 <sup>j</sup>	37.7
	60'	86	87	87	85	86	84	86 <sup>a-c</sup>	1.4	5	7	6	4	7	5	6 <sup>g-i</sup>	21.4
	30'	83	84	84	85	84	83	84 <sup>a-c</sup>	0.9	9	11	10	9	8	10	10 <sup>c-f</sup>	11.0
T 50°C	90'	86	85	83	83	85	83	84 <sup>a-c</sup>	1.6	5	6	4	7	6	5	6 <sup>g-i</sup>	19.1
	60'	84	83	81	81	83	81	82 <sup>a-c</sup>	1.6	10	8	9	10	9	8	9 <sup>fg</sup>	9.9
	30'	82	81	79	78	79	80	80 <sup>b-f</sup>	1.8	13	15	10	13	14	12	13 <sup>c-d</sup>	13.4
T 40°C	90'	80	82	80	77	81	78	80 <sup>b-f</sup>	2.3	10	12	14	11	13	14	12 <sup>d-e</sup>	13.2
	60'	77	76	78	77	77	78	77 <sup>d-f</sup>	1.0	16	14	13	17	15	16	15 <sup>c</sup>	9.7
	30'	75	74	77	76	74	76	75 <sup>e-f</sup>	1.6	21	22	21	23	22	23	22 <sup>b</sup>	4.1

Different letters between cultivars denote significant differences (LSD test,  $p < 0.05$ )

minutes (18% increase in germination). Soaking the seeds in a 75% sulfuric acid solution for 30 minutes substantially damaged the seed and improved the germination rate by a mere 4%. Concentrated sulfuric acid (98%) caused severe burns and seed mortality.

Optimal temperature treatments (exposure to either 70°C and 60°C for 90 minutes) increased the germination rate of *F. rubra* by 17%. Following pretreatment at 70°C and 60°C, 3% and 7% of the seed remained dormant, respectively. However, both temperature treatments caused both minor seed damage and incomplete termination of seed dormancy. Dormancy was broken completely after pretreatment at 90°C, although substantial seed damage occurred at 90°C. Enhancement of the germination rate was less dramatic following pretreatment at lower temperatures (40°C and 50°C) (Tab. 3). Whenever treatments were associated with high germination rates (88%-90%),

little variability in seed germination was seen between different cultivars and populations (CV = 1.2%-5.3%).

#### Sheep fescue

Following a ripening period that lasted two and a half months, dormancy of sheep fescue seeds was reduced by 5%, with no signs of an increased germination rate (Tab. 4).

Exposure of sheep fescue seed to acid and/or elevated temperatures could enhance germination rate by as much as 13%. Soaking seeds for either 10 minutes or 20 minutes in a 50% sulfuric acid solution was optimal, providing an 83% germination rate. Soaking seeds in 25% sulfuric acid for 30 minutes or exposing seeds to 80°C for 60 minutes also significantly increased germination rates to 82% ( $p \leq 0.05$ ). As much as 3%-6% of seed remained dormant following exposure to a 25% sulfuric acid solution. Exposure



to concentrated sulfuric acid (98%) resulted in complete seed mortality. At a concentration of 75%, sulfuric acid impacted seed mortality and substantially reduced the rate of germination to 42%-57%.

Seed dormancy was completely broken following pretreatment at 90°C, but this temperature treatment failed to achieve the maximum germination rate. The treatment may have caused a low level of seed mortality, especially after a 90-minute exposure to 90°C, when the germination rate was 75%. Variations in the rates of germination of different categories of sheep fescue seed were low (CV = 2.6%-6.7%) following treatments associated with maximal germination rates (82%-83%).

#### *Meadow fescue*

The highest germination rate of 93% was achieved in meadow fescue seed after soaking in a 75% solution of sulfuric acid for 30 minutes (Tab. 5). Exposure of the *F. pratensis* seeds to 90°C for 90 and 60 minutes, as well as exposure to 80°C for 90 minutes, increased germination rates to 92%. Compared with seeds of red fescue and sheep fescue, germination of seeds of meadow fescue was only promoted by exposure to higher sulfuric acid concentrations and higher temperatures, as well as by the prolonged exposure periods that were needed to achieve the highest germination rates.

Accordingly, seeds of *F. pratensis* had a higher tolerance of high concentrations of H<sub>2</sub>SO<sub>4</sub> than seeds of *F. rubra* and *F. ovina*. Accordingly, even after immersion in 98% H<sub>2</sub>SO<sub>4</sub> for either 20 minutes or 10 minutes, the respective rates of germination were 3% and 8% (Tab. 5). The acid treatment used to break seed dormancy was the most effective in promoting seed germination, with minimal seed dormancy (1%-3%) persisting when seeds were soaked for 10 minutes or 20 minutes in the lowest concentration of sulfuric acid tested. In general, the temperature treatments tested on seeds of meadow fescue had less effect on the reduction of seed dormancy than the acid treatments tested. The same effect was observed for both red and sheep fescue seeds (Tab. 3, 4, and 5).

The variability coefficient for rates of germination of meadow fescue seed was significantly lower (CV = 1.4-3.7 for treatments with germination rates of 90%-93%) compared with seeds of red fescue and sheep fescue. Thus, treatments with the highest germination of seed of cultivars and populations of *F. rubra* and *F. ovina* had higher variability in rates of germination compared with meadow fescue seed lots (Tab. 3, 4, and 5).

The tests were undertaken to identify the acid and temperature treatments capable of optimally enhancing rates of grass seed germination produced varying results. For instance, whereas rates of germination of seeds of a variety of *Brachiaria brizantha* were optimal after treatment at 75°C for 10-15 minutes (Martins and Silva, 2001), soaking in a 50% sulfuric acid solution for 25 minutes ensures optimal germination of *Cynodon dactylon* seeds, soaking in a

25% sulfuric acid solution for 15 minutes ensures optimal germination of *F. rubra* seeds, soaking in a 50% sulfuric acid solution for 10 minutes ensures optimal germination of *Lolium perenne* seeds, soaking in a 50% sulfuric acid solution for 15-20 minutes ensures optimal germination of *Poa pratensis* seeds (Salehi and Khosh-Khui, 2005), and a 4-minute exposure to a 100% sulfuric acid solution ensures optimal germination of a variety of Buffel grass (Bhattarai et al., 2008).

According to the conclusions of Stanisavljević et al. (2010a, 2010b, 2011) and Rozman et al. (2010), increased rates of germination of seeds of perennial forage grasses is accompanied by increased seedling vigour. A high rate of seed germination this creates conditions for successful establishment of grass species when competing with weeds and other species found in mixtures of seeds.

#### Conclusions

Exposure to sulfuric acid and elevated temperature enhances germination of *Festuca* seed. Optimal treatments enhanced the seed germination rate by 19% for red fescue, by 13% in sheep fescue, and by 22% in meadow fescue. Following exposure to the treatments with the highest germination rates (*F. rubra*, 88%-90%; *F. ovina*, 82%-83%; *F. pratensis*, 90%-93%), the coefficients of variability (CV) of germination were 1.2-5.3 for *F. rubra*, 2.6-6.7 for *F. ovina*, and 1.7-3.7 for *F. pratensis*. Our results may enable more successful establishment of grassland for agricultural and horticultural applications, or for preventing soil erosion.

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#### References

- Andersson L, Milberg P (1998). Variation in seed dormancy among mother plants, populations and years of seed collection. *Seed Sci Res* 8:29-38.
- Bewley JD (1997). Seed germination and dormancy. *Plant Cell* 9(7):1055-1066.
- Bhattarai SP, Fox J, Gyasi-Agyei Y (2008). Enhancing buffel grass seed germination by acid treatment for rapid vegetation establishment on railway batters. *J Arid Environ* 72:255-262.
- Bjedov I, Ristić R, Stavretović N, Stevović V, Radić B, Todosijević M (2011). Revegetation of ski runs in Serbia: case studies of Mts. Stara Planina and Divčibare. *Arch Biol Sci* 63:1127-1134.
- Casler MD (2006). Perennial grasses for turf, sport, and amenity uses: evolution of form, function, and fitness for human benefit. *J Agric Sci* 144:189-203.
- Demiroğlu G, Geren H, Kir B, Avcioglu R (2010). Performances of some cool season turfgrass cultivars in Mediterranean

- environment: II. *Festuca arundinacea* Schreb., *Festuca ovina* L., *Festuca rubra* spp. *rubra* L., *Festuca rubra* spp. *trichophylla* Gaud and *Festuca rubra* spp. *commutata* Gaud. Turk J Field Crops 15:180-187.
- Dernoeden PH (1998). Fine fescues on golf courses. Golf Course Manage 66:56-60.
- Fenner M (1991). The effects of the parent environment on seed germinability. Seed Sci Res 1:75-84.
- ISTA (2011). International Rules for Seed Testing. International Seed Testing Association, Bassersdorf, Switzerland.
- Kroumov V (2009). Natural geo-composites for grassing of eroded and degraded lands. Bull of the Serbian Geographical Soc 4:95-98.
- Martins L, Silva WR (2001). Dormancy performance of *Brachiaria brizantha* seeds submitted to thermal and chemical treatments. Pesqui Agropecu Bras 36:997-1003.
- Mitrović M, Pavlović P, Lakušić D, Djurdjević L, Stevanović B, Kostić O, Gajić G (2008). The potential of *Festuca rubra* and *Calamagrostis epigejos* for the revegetation of fly ash deposits. Sci Total Environ 407:338-347.
- Rozman V, Bukvić G, Liška A, Baličević R, Edeš A, Petrović S (2010). Differences in traits of seeds and seedlings of perennial ryegrass cultivars after nine months storage at different temperatures. Not Bot Horti Agrobo 38(1):155-158.
- Salehi H, Khosh-Khui M (2004). Turfgrass monoculture, cool-cool, and cool-warm season seed mixture establishment and growth responses. HortSci 39(7):1732-1735.
- Salehi H, Khosh-Khui M (2005). Enhancing seed germination rate of four turfgrass genera by acid treatments. J Agron Crop Sci 191:346-350.
- Simpson GM (1990). Seed dormancy in grasses. Cambridge University, London, UK.
- Stanisavljević R, Djokić D, Milenković J, Terzić D, Djukanović L, Stevović V, Dodig D (2010a). Desiccation, postharvest maturity and seed aging of tall oat-grass. Pesqui Agropecu Bras 11:1297-1302.
- Stanisavljević R, Dragičević V, Milenković J, Djukanović L, Djokić D, Terzić D, Dodig D (2010b). Effects of the duration of after-ripening period on seed germinations and seedling size in three fescue species. Span J Agric Res 8:454-459.
- Stanisavljević R, Simić A, Sokolović D (2010c). Seed production of perennial forage grasses in Serbia. Biotech Anim Husb 26:159-172.
- Stanisavljević R, Djokić D, Milenković J, Djukanović L, Stevović V, Simić A, Dodig D (2011). Seed germination and seedling vigour of Italian ryegrass, cocksfoot and timothy following harvest and storage. Cienc Agrotec 35:1141-1148.
- Tomić Z, Lugić Z, Radović J, Sokolović D, Nešić Z, Krnjaja V (2007). Perennial legumes and grasses stable source of quality livestock fodder feed. Biotech Anim Husb 23:559-572.
- Tomić Z, Bijedić Z, Vilotić D, Gačić DP (2010). Phytocenological research into the meadow associations on forest hunting grounds of Serbia. Arch Biol Sci 62:363-372.
- Tomić Z, Bijelić Z, Žujović M, Maksimović N, Stanišić N, Randelović V (2011). Floristic composition of permanent grassland in the nature park Stara Planina (Serbia). Rom Agric Res 28:187-195.
- Vintu V, Samuil C, Rotar I, Moisuc A, Razec I (2011). Influence of the management on the phytocenotic biodiversity of some Romanian representative grassland types. Not Bot Horti Agrobo 39(2):119-125.