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Short communication. Effects of the duration of after-ripening period on seed germinations and seedling size in three fescue species

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Abstract

After-ripening is a complex enzymatic and biochemical process resulting in the breakage of non-deep physiological dormancy. The experiment was set up to identify the minimum duration of storage after harvest for completion of after-ripening in meadow (*Festuca pratensis* Huds.), tall (*Festuca arundinacea* Schreb.) and red fescue (*Festuca rubra* L.). The seeds were stored under ware-house conditions for 0, 30, 60, 90, 120, 150, 180, 210 and 240 days after harvest (DAH). After the storage periods, the seeds were tested for germination and the primary root length, shoot length and seedlings biomass were measured. The final counts in all three species amounted to 60-65% germination immediately after harvest, suggesting a medium level of embryonic dormancy. The after-ripening during storage improved the final germination in all three species with decreasing intensity. In meadow fescue, the final germination was significantly improved up to 150 DAH, while the seedling size did not change significantly. In tall and red fescue, after-ripening significantly improved the final germination up to 120 DAH, while the seedling size variables reached maximum values after 150 DAH. The obtained data can serve for the determination of proper storage duration management between harvest and sowing (autumn/spring) of the tested fescue species under agro-ecological conditions of south-eastern Europe.

Additional key words: autumn and spring sowing, biomass, root length, seedling dormancy-breaking effect, shoot length.

Resumen

Comunicación corta. Efecto del periodo de post-maduración sobre la germinación de semillas y crecimiento de plántulas en tres especies de festuca

La post-maduración es un proceso enzimático y bioquímico complejo que resulta en la rotura de la latencia fisiológica poco profunda. El experimento se estableció para identificar el tiempo mínimo de almacenamiento después de la cosecha para la terminación de la post-maduración en festuca de prados (*Festuca pratensis* Huds.), festuca alta (*Festuca arundinacea* Schreb.) y festuca roja (*Festuca rubra* L.). Las semillas se almacenaron durante 0, 30, 60, 90, 120, 150, 180, 210 y 240 días después de la cosecha (DAH). Después de los períodos de almacenamiento, se analizó la germinación de las semillas y se midieron la longitud de la raíz primaria, la longitud del vástago y la biomasa de las plántulas. Las tres especies tuvieron 60-65% de germinación inmediatamente después de la cosecha, lo que indica un nivel medio de latencia de embriones. La post-maduración durante el almacenamiento mejoró la germinación final en las tres especies, con una intensidad decreciente. En *F. pratensis* la germinación final aumentó considerablemente hasta 150 DAH, mientras que el tamaño de plántulas no cambió significativamente. En *F. arundinacea* y *F. rubra* la post-maduración mejoró significativamente la germinación final hasta 120 DAH, mientras que el tamaño de las plántulas alcanzó valores máximos después de 150 DAH. Los datos obtenidos pueden servir para una gestión adecuada de la duración de almacenamiento entre la cosecha y la siembra (otoño/primavera) de las especies de festuca examinadas en las condiciones agro-ecológicas del sudeste de Europa.

Palabras clave adicionales: biomasa de plántulas, efecto de ruptura de latencia, longitud de raíz, longitud del vástago, siembra de otoño y primavera.

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Abbreviations used: DAH (days after harvest).

Meadow fescue (*Festuca pratensis* Huds.), tall fescue (*Festuca arundinacea* Schreb.) and red fescue (*Festuca rubra* L.) are worldwide important and intensively studied forage and turf species. They are widely distributed in turfs of south-eastern Europe and are often sown in seed mixtures with other species. For example, about 200,000 ha of grazing land in Bulgaria are improved pastures planted with grass-legume mixtures consisting of meadow fescue, tall fescue, and red fescue as major grasses (Fulbright, 1991). In Serbia, meadow fescue is an irreplaceable component of lawns on fertile soils and at lower altitudes, tall fescue on degraded and red fescue on wet soils and at higher altitudes (Lazarevic *et al*., 1998). According to the dry matter yield, meadow fescue (9.1 t ha^{-1}) and red fescue (7.0 t ha^{-1}) are an important source of fodder in Croatia (Leto *et al*., 2006), while tall fescue (11.5 t ha^{-1}) is in Serbia (Mihajlović *et al*., 2001). All three fescue species are characterised by a high variability of their agronomic and productive traits (Martincova, 2003; Fang *et al*., 2004; Oliveira *et al.,* 2008), which provides them with great adaptability to different environmental conditions.

Under the agro-ecological conditions of south-eastern Europe, the regular seed harvesting time is from the end of June to the beginning of July. Seeds could be sown for a new crop in autumn (August-September) two months after harvest, in the spring of the following year (March-April) eight months after harvest or even later. In perennial cereal grasses, autumn sowing assures high seed yields in the region of Strandja Mountain (Bulgaria) (Stoeva, 2005). Spring sowing is known to give a lower forage yield than autumn sowing, while seed production could be completely lacking (Stanisavljević *et al*., 2007).

Seeds of the genus *Festuca* have endogenous non-deep physiological dormancy and require a period of dry storage to trigger germination (Kaye, 1997). Storing dry seeds in an ambient (warm) environment, usually in darkness, before sowing is an *ex situ* dormancy alleviating treatment, more commonly known as «dry after-ripening» (Baskin and Baskin, 1998). Dry after-ripening may bring about changes in the levels of growth inhibitors or growth promoters within the seed embryo, encouraging seed germination (Bell, 1999). When after-ripening is completed, further seed storage induces degradation processes, such as peroxidation of polyunsaturated fatty acids, and damage of cell membranes and DNA, causing germination to decrease (Bewley and Black, 1994).

The after-ripening requirement for breaking dormancy is the result of an ecological adaptation. A six-month after-ripening requirement, as found by Goodwin *et al*. (2006) in Idaho fescue (*Festuca idahoensis*), ensures that germination coincides with the spring period, most likely to provide sufficient moisture for seedling establishment. Controlled experiments with rattail fescue (*Vulpia myuros*) indicated that fresh seeds require an after-ripening period of 1 to 12 months, depending on the germination temperature, for high levels of seed germination to be obtained (Ball *et al*., 2008). There is little information available on dormancy loss during after-ripening in other species of the genus *Festuca*. Hence, this article presents the results of an experimental study of the loss of dormancy during after-ripening in combination with seedling size variables in meadow, red and tall fescue. The implications of the results for storage management are discussed.

The experiment was set up with foundation seeds of the cultivar K-21 (meadow fescue), the cultivar K-20 (tall fescue), and the cultivar K-20 (red fescue). All three cultivars were developed in Serbia (description of the cultivars used in this study is available at http://www.ikbks.com/en/?products) and are summer active (continental cultivars). The seeds were collected at full maturity in mid-June 2007 from three-year-old production fields in the vicinity of the city Zaječar, eastern Serbia (139 m asl, 43°51'N 22°22'E). Collected seeds were cleaned manually, placed in paper bags and stored dry under standard warehouse conditions for 0, 30, 60, 90, 120, 150, 180, 210 and 240 days after harvest (DAH). After each of these storage periods, 8 replicates of 100 seeds of each variety were tested on filter paper (top of paper) for germination according to the ISTA rules (ISTA, 2008). After a prechill treatment for 5 days at 5°C, the seeds were germinated at alternating temperatures of 25/15°C (25°C for 8 h in light and 15°C for 16 h in dark). The first count of normal seedlings was made after 7 days. The final count of normal seedlings was made after 14 days for the meadow and tall fescue cultivars and after 21 days for the red fescue cultivars. In addition, the primary root length (cm), shoot length (cm) and fresh seedling biomass (root + shoot, mg) were measured after the final count. The seedling length was measured using a ruler. The moisture content of composite seed samples was measured thermogravimetrically at 130°C to constant weight (ISTA, 2008).

The data were analysed by ANOVA and differences between the means were compared by the least-signi-

ficant difference (LSD) test at the $P < 0.05$ level. To correct for non-normality of the germination percentage values, the statistical analyses were realised on arcsine transformed values. The degree of associations between the duration of the storage period and the first and final counts was estimated by multiple regression analysis (Statistica 7.0, StatSoft, Inc.).

The germination test results after different durations of storage after harvest are shown in Table 1. All three species showed a medium level of seed dormancy as immediately after harvest only between 60 and 65% germinated and many seeds remained fresh (data not shown). This is in accordance with the results obtained by Kaye (1997) who found endogenous non-deep physiological dormancy in seeds of the *Festuca* genus. Extension of the storage period resulted in a continuous increase in germination in both counts for all three fescue species. In addition, the primary root length, shoot length and seedling biomass showed increasing tendencies with increasing duration of storage, although the maximum values were not attained after the longest storage period. The results proved the requirement for after-ripening of the seeds of all three species. The harvested seeds were stored dry at about 13% moisture content under typical warehouse conditions. The mean monthly air temperatures varied from 1°C (January and February) to 22°C (August) (data not shown).

Considering meadow fescue seeds, germination did not increase significantly in both counts until 120 DAH. The major dormancy-breaking effect of after-ripening obviously appeared at 120 DAH, indicated by a sole significant increase of the final germination between two consecutive after-ripening dates (Table 1, Fig. 1). The maximum germination occurred in the first and the final count at 240 DAH (63 and 79%, respectively). Overall, there was a strong and positive relationship between the duration of the storage period and germination (R^2 was 0.954 and 0.932 for the first and the final count, respectively) (Fig. 1). The primary root length increased from 2.93 (0 DAH) to the maximum of 3.34 cm (180 DAH) and the shoot length increased from 4.86 (0 DAH) to the maximum of 5.27 cm (150 DAH). The seedling biomass increased from 11.76 (0 DAH) to the maximum of 12.19 mg (150 DAH). Nevertheless, the differences in all three seedling size variables were not significant. Rapid seedling growth is an

Table 1. Seed germination (first and final count) and seedling size variables (root length, shoot length and biomass) after various lengths of dry storage in meadow, tall and red fescue seeds

Character		After-ripening in days									LSD 5%
		0	30	60	90	120	150	180	210	240	
Meadow fescue											
Germination (%)	First count	34	34	36	38	41	48	51	54	63	6.27
	Final count	60	60	62	63	70	73	74	76	79	6.11
Length (cm)	Shoot	4.86	4.86	4.91	4.93	5.23	5.27	5.23	5.23	5.23	0.59
	Root	2.93	2.98	3.05	3.12	3.22	3.22	3.34	3.27	3.19	0.43
Seedling biomass (mg)		11.76	11.88	11.97	12.01	12.03	12.07	12.19	12.16	12.14	0.81
Tall fescue											
Germination (%)	First count	34	39	47	52	67	70	73	74	77	5.55
	Final count	65	69	75	77	89	90	91	91	89	5.12
Length (cm)	Shoot	4.90	4.90	4.91	4.95	5.27	5.29	5.33	5.28	5.30	0.39
	Root	3.49	3.50	3.51	3.51	3.53	3.77	3.87	3.87	3.76	0.32
Seedling biomass (mg)		11.96	12.08	12.17	12.23	12.36	12.75	12.69	12.61	12.53	0.78
Red fescue											
Germination (%)	First count	40	42	45	47	62	70	74	77	79	6.70
	Final count	61	64	67	78	82	83	84	84	84	6.62
Length (cm)	Shoot	3.81	3.93	3.94	3.96	3.92	4.07	4.10	4.10	4.26	0.29
	Root	1.96	1.98	2.00	2.01	2.10	2.17	2.24	2.25	2.04	0.22
Seedling biomass (mg)		6.40	6.47	6.68	7.11	7.40	7.47	7.22	7.35	6.55	0.58

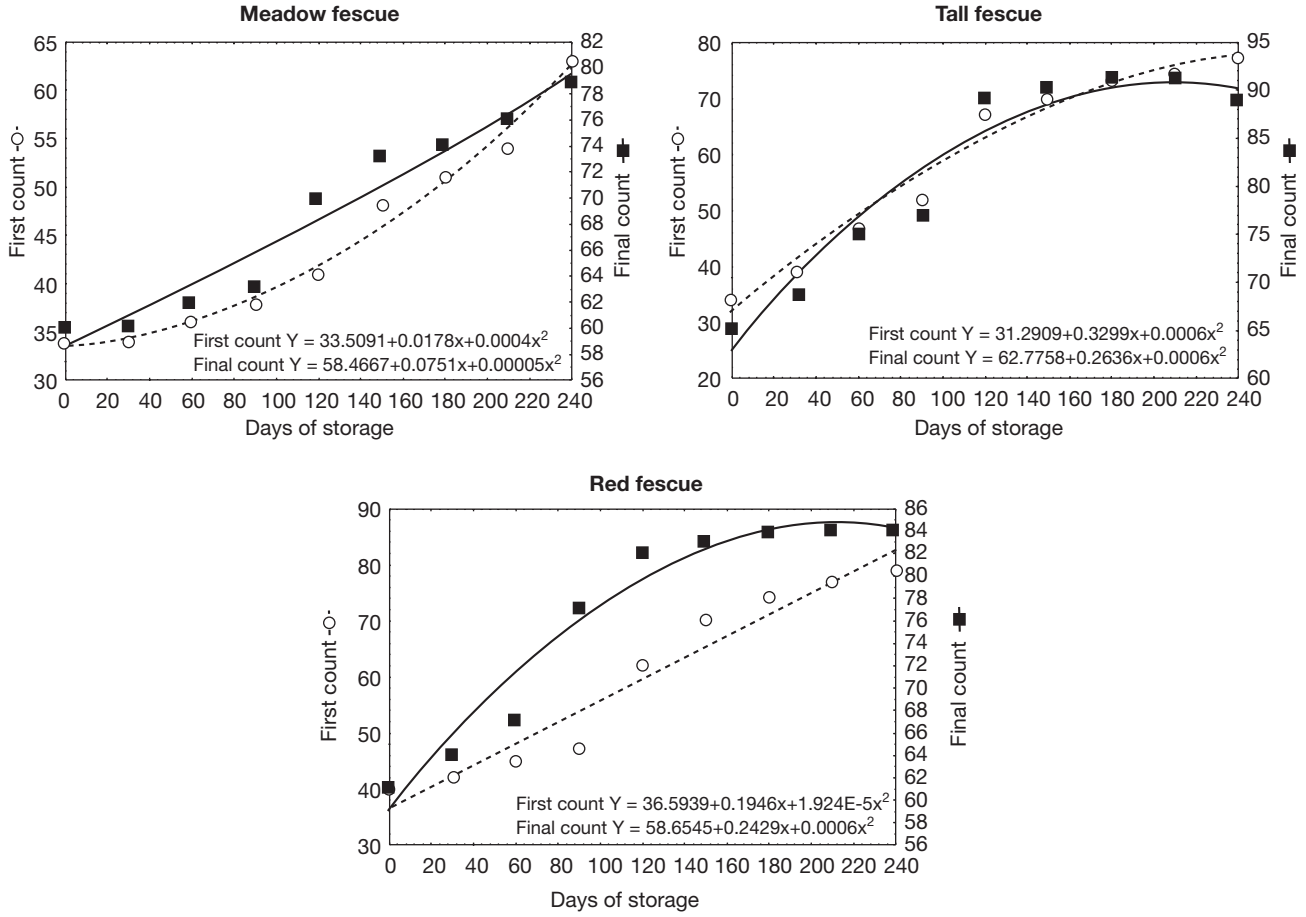

Figure 1. Relationship between the duration of storage and seeds germination (first and final count) in meadow, tall and red fescue.

essential phase of turf grass culture and is a key attribute for weed competition and a rapid turf cover. The seed-producing capacity of two varieties of meadow fescue, S215 and S53, correlated well with the rapidity of seedling establishment (Lewis, 1968).

Considering tall fescue seeds, a significant germination increase occurred between 30 and 60 DAH (8 and 6% for the first and the final count, respectively), indicating the start of dormancy loss (Table 1, Fig. 1). Thus, tall fescue seeds began to lose dormancy 30 and 60 days earlier than red and meadow fescue seeds, respectively. The after-ripening period was completed at 180 DAH, when the maximum germination occurred. For Idaho fescue seeds, Goodwin *et al*. (2006) also found that a six-month after-ripening was required to obtain maximum germination. There was a significant positive relationship between the storage period and germination (R^2 was 0.935 and 0.835 for the first and the final count, respectively) (Fig. 1). Dudeck and Peacock (1986) reported that storage at higher temperatures accelerated after-ripening in tall fescue seeds. One month after harvest, the mean germination of cv. K-20 seeds was 69%, which was a little higher than 63% for cv. Demeter reported by Lodge (2004). Tall fescue germinates rapidly without pre-treatment as it lacks a physiological dormancy (Hill *et al*., 1985; Lodge, 2004). Contrary to meadow fescue, the seedling proportions were affected by after-ripening. The primary root length increased significantly from 4.90 cm (0 and 30 DAH) to 5.33 cm (180 DAH) and the shoot length from 3.49 cm (0 DAH) to the maximum of 3.87 cm (180 and 210 DAH). The seedling biomass significantly increased from 11.96 mg (0 DAH) to the maximum of 12.75 mg (150 DAH).

With red fescue seeds, the first count germination constantly increased up to 240 DAH but this increase was no longer significant after 180 DAH (Table 1). The final count germination significantly increased, by 11%, between 60 and 90 DAH (Table 1, Fig. 1), but thereafter, there was no significant increase. There was

a significant positive relationship between the duration of the storage period and germination (R^2 was 0.943 and 0.844 for the first and the final count, respectively) (Fig. 1). A similar result for the number of days (90) required for dormancy loss for *Puccinellia distans* (L.) Parl. seed was reported by Tarasoff *et al*. (2007). The primary root length increased significantly from 3.81 (0 DAH) to 4.26 cm (240 DAH) and the shoot length from 1.96 (0 DAH) to the maximum of 2.25 cm (210 DAH). Although the primary root and shoot lengths were not significantly improved until 180 DAH, the biomass already showed a significant increase at 120 DAH up to the maximum of 7.47 mg (150 DAH).

The majority of the south-east European region is rainfed and belongs to temperate continental climatic zone. Late summer-early autumn and early spring are the two periods for sowing fescues in this region. The decision on autumn or spring sowing is based on local experience (location, soil type, rainfall/temperature patterns, etc.). For example, in dryland dairying, autumn sowing is preferred as it provides the maximum time for establishment before the onset of hot, dry summer weather (Burnett, 2008). Early autumn sowing is also preferred by turf experts because it allows for more growing time under ideal growth conditions (autumn and spring growing season). Spring sowing is considered the second best sowing date. The disadvantages of spring sowing may include late germinating weed species and a drying soil profile.

High germination and rapid seedling development are prerequisites for successful turf establishment. Therefore, the patterns of germination and of the seedling size variables measured in this study provide another aspect for deciding about the best sowing date and, in particular, about the minimum time period between harvest and sowing of meadow, tall and red fescue. The percentage germination of meadow fescue seeds increased only after 120 DAH. As fescue seeds are harvested in south-eastern Europe mainly in June, germination of at least 70% and rapid growth may not be provided for before the beginning of November. Sowing between November and March is not practicable because of low soil temperatures and frost. Thus, early spring (April) sowing seems to be the best sowing period for freshly harvested meadow fescue seed. A satisfactory final germination of 70% was attained for red fescue between 60 and 90 DAH, corresponding to mid-August to mid-September. The seedling biomass had maximum values in mid-October and mid-November (120 and 150 DAH, respectively). Thus, the obtained data suggest late August to mid-October as the most suitable period for red fescue sowing. Rather similar patterns of germination and seedling size were detected for tall fescue. Tall fescue seeds began to lose dormancy at 60 DAH (mid-August) and reached a very high level of germination (89%) by 120 DAH (mid-October). Thereafter, seed germination and the seedling size variables decreased only slightly up to 240 DAH (mid-February), enabling this species to be considered for spring sowing also. In some areas, autumn sowing of tall fescue can be problematic because of low soil temperatures and moisture variability. Where these conditions occur, the advantages of spring sowing of the Continental cultivars (summer active) of tall fescue include better weed management, full profile of soil moisture and rising temperatures (Burnett, 2008). In practice, there may be also interest of storing seeds for the next autumn sowing. In extension of this research, final germination after 420 DAH was still high in meadow, tall and red fescue (76%, 84% and 80%, respectively). In case of good storage conditions, this may allow good growing conditions in the field to be combined with high seed quality.

In conclusion, after-ripening improved the final germination in all three species with a decreasing degree. A significant dormancy-breaking effect was found at 60, 90 and 120 DAH for tall, red and meadow fescue, respectively. At least five months of after-ripening were required before maximum germination and seedling size variables were obtained for all fescue species. The results obtained in this study indicated that in south-east Europe, from the quality perspective, early autumn is the best sowing period for freshly harvested seeds of red and tall fescue while early spring is the best for meadow fescue.

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