

Research Paper

Effect of seed storage on seed germination and seedling quality of *Festulolium* in comparison with related forage grasses

Efecto del almacenamiento de la semilla de Festulolium y especies relacionadas en su germinación y la calidad de plántulas

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Abstract

Tests of seed germination, seed dormancy and seedling growth were performed on 0-, 6-, 20- and 30-months-old seed lots of *Festulolium* in comparison with Italian ryegrass (*Lolium multiflorum*) and meadow fescue (*Festuca pratensis*). Tests were performed on seeds harvested in 2 different years (2014 and 2015) resulting in no major difference between the years. Seed storage affected seed viability and dormancy and seedling growth in all 3 grasses. The maximum germination of *Festulolium* seeds was achieved 6 months after harvest (95% normal seedlings); germination decreased significantly thereafter. While maximum germination of *L. multiflorum* and *F. pratensis* seeds was also achieved following storage for 6 months, these germination rates (93 and 90%, respectively) were retained until at least 20 months in storage. After storage for 30 months, seed germination of *Festulolium*, *L. multiflorum* and *F. pratensis* had declined to 72, 79 and 83%, respectively. High germination in all species was associated with higher rates of seedling growth. In an artificial seed ageing test, a temperature of 41 °C (during 48 and 72 hours) was found to effectively rank seed lots for germination performance in all 3 grasses. This test seems to have application for use in the seed trade to identify seed lots which could deteriorate more rapidly in storage. Further studies are needed to verify this hypothesis.

Keywords: Ageing of seed, ageing test, dormancy, embryonic stem and radicle, forage grasses.

Resumen

Se realizaron pruebas de germinación y de crecimiento de plántulas provenientes de lotes de semillas de *Festulolium* almacenadas durante 0, 6, 20 y 30 meses, en comparación con raigrás italiano (*Lolium multiflorum*) y festuca de pradera (*Festuca pratensis*). En lotes de semillas cosechadas en 2014 y 2015 no se encontraron diferencias entre los años. El almacenamiento afectó la viabilidad y la latencia de las semillas y el crecimiento de las plántulas en las tres especies. La germinación máxima de las semillas de *Festulolium* se presentó 6 meses después de la cosecha (95% de plántulas normales), a partir de los cuales disminuyó significativamente. También las semillas de *L. multiflorum* y *F. pratensis* presentaron máxima germinación después de 6 meses (93 y 90%, respectivamente); estas tasas, sin embargo, se mantuvieron hasta al menos 20 meses de almacenamiento. Después de 30 meses, la germinación de las semillas disminuyó a 72, 79 y 83% para *Festulolium*, raigrás y festuca, respectivamente. La alta germinación en todas las especies se asoció con mayores tasas de crecimiento de plántulas. En una prueba rápida de envejecimiento artificial de semillas (temperatura de 41 °C durante 48 y 72 horas) fue posible predecir el comportamiento de germinación de las semillas de las tres especies. Esta prueba parece tener aplicación en el comercio para identificar lotes de semillas que podrían deteriorarse más rápidamente durante el almacenamiento. Se necesitan más estudios para verificar esta hipótesis.

Palabras clave: Dormancia, envejecimiento de semilla, gramíneas forrajeras, prueba de envejecimiento, radícula y tallo embrionarios.

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Introduction

Lolium multiflorum, *L. perenne*, *Festuca pratensis* and *F. arundinacea* are widely cultivated forage grasses and have been studied from the aspects of genetics and breeding, seed production, growing practices and utilization. Work on interspecies hybridization between *Lolium* and *Festuca* was conducted in the 1970s, and a novel hybrid named *Festulolium* was recorded in Europe after 2010 (Østrem et al. 2013). According to Humphreys et al. (2013; 2014), the hybrid grass *Festulolium* was a result of crosses of *F. pratensis* or *F. arundinacea* with *L. perenne* or *L. multiflorum*. As stated by Akgun et al. (2008), *Festulolium* is characterized by higher yields of green fodder and dry matter than those of *L. multiflorum* and *F. pratensis*, improved resistance to environmental stresses (Abdelhalim et al. 2016) and improved fodder quality (Skládanka et al. 2010). In addition, it displays a different pattern of plant growth in the autumn-early winter period; e.g. × *Festulolium braunii* expands its root apical meristem (RAM) and continues to grow, while cellular growth in the RAM of *F. pratensis* declines and reduces vegetative growth (Pašakinskienė and Švėgždienė 2018). Furthermore, seed yields recorded for *Festulolium* have been superior to those of *L. multiflorum* and *F. pratensis* (Akgun et al. 2008). Optimum growing systems have been developed for the cultivation of *Festulolium* for seed (Deleuran et al. 2010).

While *Festulolium*, *Festuca arundinacea* and *L. perenne* are considered temperate grasses, in a subtropical climate (central Mexico) analyses of forage quality and milk production in feeding studies with dairy cows showed these species compared favorably with kikuyu grass (*Cenchrus clandestinus* syn. *Pennisetum clandestinum*) (Plata-Reyes et al. 2018). Likewise Mwendia et al. (2019) concluded that perennial ryegrass and festulolium have the potential to contribute to improving the forage resource base in the highlands of Central Kenya and similar areas.

Fodder manufacturers and seed companies have enquired about the relationship between seed quality and

establishment of *Festulolium* in combination with legumes or as a monocrop. Moreover, seed companies and seed trade companies are concerned about maintenance of seed quality during storage. Literature searches revealed no studies on changes in *Festulolium* seed quality during the storage period (seed ageing).

In the absence of previous research, studies reported in this paper were conducted to observe changes in seed germination, dormancy and seedling vigor in *Festulolium*, *L. multiflorum* and *F. pratensis* over 30 months of storage.

Materials and Methods

Seeds were sampled from seed crops grown near the city of Smederevo, Serbia (44°40'–44°66' N, 20°56'–20°93' E; 66–98 masl). Three seed production lots (I, II and III) were used for each species tested. All seed lots were harvested with a small combine in June 2014 and in June 2015. After harvest, the cleaned seeds were dried to moisture content below 14% and stored under ambient conditions for 30 months (Table 1). Seeds of the following species were collected: 'Perun' *Festulolium* (*Festulolium*), 'Kruševački 21' meadow fescue (*Festuca pratensis*) and 'Kruševački 13' Italian ryegrass (*Lolium multiflorum*). Storage conditions during the investigation period are shown in Table 1.

Kruševački 21 was produced by breeding genotypes originating from indigenous populations of eastern Serbia and Resava. Plant height is about 105 cm and tillering is strong, while resistance to rust disease (*Puccinia* sp.) is enhanced. The semi-erect shoots are characterized by a clear pattern of light green leaves and a dark green fine stem. The seed is medium-sized and uniform, with 1,000-seed weight of approximately 1.96 g. Under conditions of continuous cropping, it produces 9–10 t DM/ha with 160 g CP/kg at a sowing rate of 20–25 kg/ha. It is recommended for all types of long-lasting grass and clover-grass mowing mixtures.

Table 1. Average monthly temperatures (°C) and relative humidity (%) during storage of up to 30 months of *Festulolium*, *Festuca pratensis* and *Lolium multiflorum* seeds harvested in June 2014 (period of seed storage: June 2014–November 2016) and June 2015 (period of seed storage: June 2015–November 2017).

Year of storage	Variable	Jan	Feb	Mar	April	May	June	July	Aug	Sep	Oct	Nov	Dec
2014	T						20.6	22.4	22.2	12.4	11.0	6.0	3.0
	RH						64.0	64.3	68.5	74.5	76.4	77.7	79.5
2015	T	2.1	6.2	9.2	11.3	13.8	20.5	23.1	24.1	13.0	11.2	6.2	3.2
	RH	81.0	79.2	78.5	66.1	65.6	63.2	64.8	68.6	74.7	76.7	77.4	79.6
2016	T	2.3	6.3	9.3	11.4	13.7	20.5	22.7	23.6	12.7	11.1	6.0	3.3
	RH	81.8	79.4	78.6	66.3	65.9	63.6	64.7	68.5	74.6	76.6	77.5	79.6
2017	T	2.3	6.3	9.3	11.4	13.8	20.4	22.5	23.1	12.5	11.2	6.2	
	RH	81.5	79.3	78.5	66.3	65.8	63.7	64.5	68.3	74.4	76.8	77.3	

T - Temperature; RH - Relative humidity.

Kruševački 13 was developed by breeding and selection of genotypes of introduced populations. The height of regenerative shoots in the first cutting reaches 1 m, while vegetative shoots are about 80 cm tall. The shoots are covered with many broad, distinctly glossy leaves of clear green color. The plants tiller strongly and produce medium tender, greyish green stems. The spikes are medium in length with 6–8 spikelets, while seeds are easily shed and have a 1,000-seed weight of 1.99 g. The crop is high-yielding and under favorable conditions can produce in excess of 14–15 t hay/ha with 16% CP. It is an excellent variety for sowing in mixtures with red clover for 3-year leys, is very productive under irrigation and is also suitable for early spring and late autumn utilization.

Seed testing was performed with 2 experiments: Experiment 1: testing of germination and seedling growth (embryonic stem, radicle, seedling weight); and Experiment 2: seed ageing test. Both experiments were performed on each seed lot (I, II and III) according to standard methodology. Given that there were no significant differences in germination and seedling growth between seed lots, results for individual seed lots are not shown. However, in the ageing test, differences in germination between seed lots were significant. This is significant in practice when deciding which seed lot can stay longer in a warehouse, and which needs to be packaged for market earlier.

Experiment 1: Seed germination and dormancy; seedling growth

Germination tests were performed after 0, 6, 18 and 30 months of storage. After being chilled at 5 °C for 5 days, seeds were sown in boxes filled with sand (20 × 14 × 4 cm). The boxes were then placed into germination cabinets (temperature 20/30 °C; 8 h light at 1,520 lux and 16 h dark; 50 seeds for each of 3 replications). Multiple incubators were employed and species were uniformly distributed. Seed germination and dormancy were determined on day 14 after sowing. Seed was considered to have germinated when a radicle and embryonic stem up to 1 cm had developed. The tetrazolium test was applied to distinguish dormant seeds from dead seeds (ISTA 2019) after 14 days. In addition, primary root length, shoot length and fresh seedling biomass (root + shoot) were measured after the final count. Seedling length was measured using a ruler (Stanisavljević et al. 2011).

Experiment 2: Ageing test

A seed ageing test was applied to a subsample of 6-months-old seeds for each species and each seed lot using 4 replications of 100 seeds that were evenly arranged on the

top of a box screen (11 × 11 × 3.5 cm) containing 40 mL of distilled water, which provided a relative humidity of 98–100%. Grid boxes were used for this test to keep the seeds moist but not submerged in water. The boxes were placed in a water bath at 41 °C for 48 and 72 h. Seed moisture contents before the test and after 48 and 72 h were determined with values of 12.0–12.7% before placing in the water bath and 36.6–37.5% after being in the water bath, which is in accordance with ISTA recommendations (Hampton and TeKrony 1995). This test was performed according to the method applied for the evaluation of seed lots of *L. multiflorum* (Tunes et al. 2011) and *Festuca pratensis* (Stanisavljević et al. 2013). We were unable to locate any data in the available literature regarding the application of the ageing test to *Festulolium* seeds – data are lacking on appropriate temperature and duration of seed subjection and seed moisture prior to testing. Following exposure to this temperature (41 °C) and duration (48 and 72 h), a germination test was performed as described above.

Statistical analysis

The experimental data were analyzed using 3-way factorial analysis of variance (ANOVA). Tukey's multiple range F test and coefficient of variation were used to test for the effects of the treatments. Standard error of the mean was calculated to indicate variation around the mean. Data for germination and dormancy percentages were arcsine transformed [$\sqrt{x/100}$] before being subjected to analysis of variance. The program Minitab, version 16.1.0 (Minitab Inc., State College, PA, USA) was used to process data (free version).

Results

Since there were no significant differences for seed germination and seedling growth between the seed lots tested or for the harvest years 2014 and 2015, data for both years were combined and means for each species and treatment are presented (Tables 2 and 3). Seed age (length of time in storage) was the only parameter which affected germination percentages of seed of the 3 species and development of seedlings. Seed age significantly affected germination and initial growth of seedlings for *Festulolium* ($P \leq 0.001$), plus *L. multiflorum* and *F. pratensis* ($P \leq 0.01$ or $P \leq 0.05$) (Table 4).

Experiment 1 (seed germination and dormancy; seedling growth)

Seed germination and dormancy. Immediately after seed drying (0 months), germination of *Festulolium* seeds was higher by 7 and 4% than that of *F. pratensis* and

Experiment 2 (ageing test)

Application of the seed ageing test to the 6-months-old seeds showed differences in seed germination (%) between seed lots for all 3 species tested. Whereas the classical germination test did not show a significant difference (F test) between the seed lots of any of the tested species, the aging test was able to detect differences between seed lots in germination of all tested species (Table 4). The test also showed a faster decline in germination percentage (average for all 3 seed lots) of *Festulolium* seed (from 55% after 48 h to 17% after 72 h) than of *L. multiflorum* seed (from 76 to 53%) and *F. pratensis* seed (from 83 to 73%) (Table 4).

Table 4. Germination percentages (\pm s.e.m.) of 6-months-old seed lots of *Festulolium*, *Lolium multiflorum* and *Festuca pratensis* after an ageing test by placing in a water bath at 41 °C for 48 and 72 hours.

Species	Seed lot	Test duration	
		48 h	72 h
<i>Festulolium</i>	I	52 \pm 0.45b	18 \pm 0.27b
	II	68 \pm 0.39a	21 \pm 0.29a
	III	45 \pm 0.37c	13 \pm 0.51c
	Mean	55	17
<i>L. multiflorum</i>	I	77 \pm 0.29b	53 \pm 0.32b
	II	81 \pm 0.63a	59 \pm 0.39a
	III	71 \pm 0.43c	48 \pm 0.77c
	Mean	76	53
<i>F. pratensis</i>	I	86 \pm 0.37a	79 \pm 0.28a
	II	79 \pm 0.44 b	66 \pm 0.36c
	III	83 \pm 0.51ab	74 \pm 0.41b
	Mean	83	73

Values within columns and species with different letters are significantly different ($P < 0.05$) by Tukey's multiple range test.

Discussion

Experiment 1

Observed seed lots were from regions geographically close and harvested in a similar way, which may be a reason for their uniformity in terms of tested traits of seed and seedling quality.

While germination rates of seed of all species was quite acceptable following harvest, the declining germination after storage is potentially an issue for the seed trade, because the minimum legislated germinations of *F. pratensis* and *L. multiflorum* seeds at sale are 75 and 70%, respectively. Following 30 months of storage, germination rates for *Festulolium* and *L. multiflorum* seeds were 72 and 79%, respectively, so seed ageing

under ambient conditions was an important issue. Seed dormancy immediately after harvest or dispersal under natural conditions is a biological trait that prevents germination of some seeds as a survival mechanism in the event that seedlings cannot cope with environmental conditions following germination (Bewley 1997). This trait is influenced by multiple factors including genetics, physiology, biochemistry and histology (Graeber et al. 2012; Long et al. 2015; Sah et al. 2016).

Dormant seeds of plants can reduce germination percentage at any one time (Stanisavljević et al. 2014). For farming systems, where synchronized germination is desired, farmers sometimes compensate for dormancy by increasing sowing rates (an additional cost) to achieve optimum populations for high yields. An alternative approach is to treat seed prior to sowing, e.g. by scarification or heat treatment, to break dormancy and ensure more uniform germination. This is particularly important for species with high percentages of dormant seeds at harvest, e.g. legume seeds.

While seedlings developed from dormant seeds would normally be healthy, the delay in their emergence and development can reduce their capacity to compete with already developed seedlings (seedlings developed from immediately germinated seeds) (Bass et al. 1988). According to Adkins et al. (2002), seed dormancy is common in bred forage grasses, especially soon after harvest, unlike many cereal crops, where breeding has been used to reduce seed dormancy. When studying dormancy in *F. pratensis*, Stanisavljević et al. (2012) found low variability (CV = 3.6%) among 3 varieties and 3 populations immediately after harvest but further studies showed that, depending on seed moisture content at harvest, seed dormancy varied from 33 to 18% (Stanisavljević et al. 2013). Dormancy percentages in our current study following harvest ranged from 10% (*Festulolium*) to 17% (*F. pratensis*).

After the maturation period, the dormant seeds become germinable, total germination increases and at that stage the utilizable value of seeds is at its peak, i.e. the desired number of plants can be obtained from sowing lower amounts of seeds – the most economic establishment of crops. According to Stanisavljević et al. (2011), a storage period of 500 days under uncontrolled temperature and humidity conditions is necessary for *L. multiflorum* seeds to mature and achieve maximum germination. The current study suggested that storage for 180 days was sufficient to reach maximum germination percentage. Being able to market and plant seed at 180 days following harvest rather than 500 days after harvest is much more cost-efficient. While germination of *Festulolium* declined from this time, *L. multiflorum* (92–90%) and *F. pratensis* (90–92%)

remained at peak levels until 20 months of storage (Table 2), showing a small advantage for these species.

A significant advantage of sowing non-dormant seeds is faster and more uniform initial growth of the established crop. In our studies, an additional outcome with high germination was stronger growth of the embryonic stem and radicle, as well as increased weight of seedlings (Table 3). These factors are very important because mixtures of forage grasses are commonly grown (Wyszkowska et al. 2019), sometimes with forage legumes as a mixed crop (Neuberg et al. 2011).

As demonstrated in this study, the stage of maximum germination is followed by ageing of the seed, which involves irreversible changes to seeds, ultimately resulting in decreased capacity to germinate and grow as a seedling. In these studies, the most pronounced ageing was observed in *Festulolium* (germination was 72% after 30 months). Long-term storage of this species is not recommended.

Seed ageing is specific for each plant species (Nagel and Borner 2010). The consequence of seed ageing is not just a germination reduction but also a reduction in the initial growth of the radicle, embryonic stem and seedling weight (Rajjou et al. 2008; Stanisavljevic et al. 2011). A seed ageing test measures the ability of a seed lot to resist degradation changes and protection mechanisms that are present in the seed (Balešević-Tubić and Tatić 2012). According to Hare et al. (2018) germination percentage under laboratory conditions declined to below 50% after 3 years of storage of seed for 2 guinea grasses (*Megathyrsus maximus* syn. *Panicum maximum*) cvv. Mombasa and Tanzania, 4 years for Ubon paspalum (*Paspalum atratum*) and 4–5 years for *Urochloa* syn. *Brachiaria* hybrid cv. Mulato II seed.

Experiment 2

The difference between seed germination in Experiment 1 and germination in the seed ageing test is noteworthy as are the differences between the 3 seed lots in the seed ageing test. This should be considered as evidence of the sensitivity of the ageing test. According to Marcos Filho (2015), the seed ageing test is one of the most applicable tests for determining how well seeds survive under storage. Its application in forage seed testing is less significant than in vegetable seeds (Wang et al. 2004). The reason for this is probably that expensive hybrid seeds and individual seed sowing are used in the production of vegetable and some field crops. It is important to ensure that expensive seeds do not decay before sale, so this quick test is a good way to evaluate longevity. In our case, the ageing test proved useful as a

rapid test which provided us with information about how seed lots might perform after lengthy storage and estimates of the deadlines for use of certain seed lots to prevent serious deterioration in germination percentage. Prolonged exposure to adverse conditions during the ageing test inevitably leads to loss of quality and reduced germination (Marcos Filho 2015). In our experiment, by applying the 2 seed ageing test durations, germination of *Festulolium* decreased from 42 to 72 h test duration by 38%, *L. multiflorum* by 23%, and *F. pratensis* by 10% (Table 4). Tunes et al. (2011) reported that a seed ageing test performed on 4 seed lots of *L. multiflorum* with an increased duration of 72 vs. 48 h reduced germination by an average of 38%. The recommended temperatures for the ageing test and the length of seed exposure vary by forage species: for *Brachiaria brizantha*, Hernández et al. (2017) recommended 40 °C during 48 or 60 h. For *L. multiflorum*, the recommended temperature is 41 °C and exposure time 24, 48 or 72 h, depending on whether a traditional test or an alternative test with NaCl is performed (Tunes et al. 2011). In *F. pratensis* the recommended temperature is 41 °C and exposure time 48 or 72 h (Stanisavljević et al. 2013).

Conclusions

i) According to our results *Festulolium* seeds stored under ambient conditions with seeds of *Lolium multiflorum* and *Festuca pratensis* generally had lower seed dormancy and a shorter period was necessary for post-harvest maturation, release from dormancy and for achieving maximum germination. However, *Festulolium* seeds were less resistant to the ageing process, and germination percentage deteriorated more rapidly with time in storage than for seeds of *L. multiflorum* and *F. pratensis*. Whether this pattern would be changed with control of storage conditions remains unanswered.

ii) High germination performance was accompanied by vigorous growth of seedlings of all species, providing an added benefit with good quality seed.

iii) The seed ageing test proved a simple and rapid test to provide estimates of how germination of seed samples would change over extended periods of storage. More widespread use of this simple test commercially should identify seed lots which could deteriorate rapidly in storage. Further testing is needed to verify this hypothesis.

Acknowledgments

We thank the Ministry of Education, Science and Technological Development, Republic of Serbia, for financial support.

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(Note of the editors: All hyperlinks were verified 24 April 2020.)

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(Received for publication 25 September 2019; accepted 31 January 2020; published 31 May 2020)

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