

## High-temperature effects on seed germination of fourteen Kentucky bluegrass (*Poa pratensis* L.) cultivars

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**Abstract.** Kentucky bluegrass (*Poa pratensis* L.) is a perennial cool-season grass commonly used for sport and ornamental turfgrasses in transition zones. It is a rather difficult species to establish due to slow germination and the relatively moderate growth rate of seedlings. Early autumn is considered the best time for sowing Kentucky bluegrass in temperate regions. Spring sowing is not recommended as low soil moisture and high temperatures can have a negative impact on germination. However, unavoidable circumstances often force turfgrasses to be sown in spring with high probability of failure. The risk of failure may increase in the near future as a consequence of climate change, so more knowledge is required on the ability of Kentucky bluegrass cultivars to germinate at high temperatures. A laboratory study evaluated the germination response of fourteen cultivars selected among those most used in northern Italy. They were compared in a conditioning chamber under five regimes of alternating temperatures (20/30 °C, 23/33 °C, 26/36 °C, 29/39 °C, 32/42 °C). Germination was recorded weekly starting from sowing. The germination patterns were similar up to 26/36 °C. At 29/39 °C only five cultivars had a germination of over 50%. At the highest temperature regime none of the cultivars had more than 3% germination. It is concluded that only when very extreme high temperatures occur, growers need to pay attention to the choice of cultivars to avoid problems during the germination-emergence phase, but based on the climate change scenario this is likely to happen with greater frequency in the future.

**Key words:** germination pattern, climate change, germination temperature, turfgrass, establishment.

### INTRODUCTION

Kentucky bluegrass (*Poa pratensis* L.) is a perennial cool-season grass native to Europe and Asia widely distributed throughout the cool humid regions where it is commonly used for establishing sport and ornamental turf (Hartley, 1961; Huff, 2003; Raggi et al., 2015). This species is appreciated because it combines high aesthetic value with the ability to recover quickly from damage by means of vigorous creeping rhizomes (Bradley et al., 2010; Bonos & Huff, 2013). Although Kentucky bluegrass can be

established vegetatively from rhizomes, the primary method of establishing turfgrass is from seed.

The establishment rate of Kentucky bluegrass is slower than most cool-season species, due mainly to slow germination that takes approximately 2 weeks in soil and low seedling growth rate (Hall, 1996; Brede, 2000; Huff, 2003; Ball, 2009). The slow germination of this species may be due to a higher thermal time (or hydrothermal time) required for the process (Larsen & Bo Martin, 2005). Early autumn is the ideal time for sowing Kentucky bluegrass in transition zones because soil temperature and moisture are generally stable and sufficiently high to ensure germination. Spring sowing is more problematic, especially because soil temperatures vary significantly during the season, however, circumstances often warrant a spring seeding. Due to climate change spring temperatures not favourable for germination of cool-season species are expected to occur more frequently than in the past. This scenario is confirmed by numerous studies that predict an increase in summer days (when daily Tmax is above 25 °C) and tropical nights (when daily Tmin is above 20 °C) for Italy; a lower cumulative precipitation is also expected by simulation models for all Italian regions (Toreti et al., 2008; Medri et al., 2013; IPCC, 2014; Zollo et al., 2015). The Mediterranean region is foreseen to be particularly exposed to possible exceptional temperatures, increasingly frequent extreme weather events and reduced annual precipitation over the coming years (Monai et al., 2010; Medri et al., 2013; Mazdiyasi & AghaKouchach, 2015). The high temperature hardiness of Kentucky bluegrass has been widely studied (Wehner & Watschke, 1981; Minner et al., 1983; Jiang and Huang, 2001; Su et al., 2007), but little information is available on the effects of high temperatures on germination. Larsen et al. (2004) observed that germination percentage of the cultivars *Andante* and *Brodway*, assessed in the laboratory, was lower at 25 °C than 10 °C and pointed out suggested that the optimum temperature for germination is below 25 °C. In a growth chamber experiment Van't Klooster (2007) found significant differences in temperature germination response among Kentucky bluegrass cultivars under fourteen temperature levels ranging from 5 to 35 °C. Aamlid & Arntsen (1998) tested germination of Kentucky bluegrass under several temperature and continuous light regimes on a thermogradient plate, on paper and in soil in a phytotron, and demonstrated that temperature and cultivar were the main factors driving the germination process.

Since improved cultivars are developed continuously by turfgrass breeders, information on the germination capacity of new materials under high temperature conditions seems to be essential. The objective of this study was therefore to evaluate the germination response to extreme high temperatures of fourteen cultivars of Kentucky bluegrass with the aim of helping turf managers in cultivar selection to successfully establish turfgrasses from seed.

## MATERIALS AND METHODS

A study was done at the laboratory of the Agricultural Research Council in Tavazzano (Lodi), Italy, to define the germination response of fourteen Kentucky bluegrass cultivars at five levels of alternating temperatures. The experiment was conducted during spring 2015 in conditioning chambers (mod. CRIOCABIN 2004, Cavallo srl, Milan). The Kentucky bluegrass cultivars compared were 'Balin', 'Barimpala', 'Blue Sapphire', 'Brooklawn', 'Evora', 'Geronimo', 'JumpStart',

‘Marauder’, ‘Mercury’, ‘Moonlight Slit’, ‘Nublue Plus’, ‘Platini’, ‘Right’ and ‘Sobra’. These cultivars were chosen on the basis of their current availability on the Italian turfgrass market. The seeds were pre-chilled at a constant temperature of 5 °C for 5 days according to ISTA rules. Six replicates of fifty seeds for each of the cultivars were then incubated at five levels of alternating temperatures: 20/30, 23/33, 26/36, 29/39, 32/42 °C and photoperiod of 8h darkness/16h light. Irradiance was provided by cold white fluorescent tubes at 880 lux m<sup>-2</sup> during the higher temperature period. In each replicate, the seeds were placed on filter paper saturated with deionized water (4.0 ml) in glass Petri dishes (11 cm diameter). Additional deionized water was added weekly as needed to keep the filter paper moist. The experimental design was completely randomized. Germinated seeds were counted weekly. The experiment was stopped after 28 days, when germinated seeds were no longer observed.

### Data analysis

#### *Germination patterns and t<sub>50</sub>*

The germination pattern was analyzed using a logistic function in the Bioassay97 program (Onofri 2001) as follows:

$$GP = \frac{M}{1 + e^{a \cdot (\ln(t+0,0000001) - \ln b)}} \quad (1)$$

where GP is the germination percentage on total sown seeds,  $M$  is the percentage of maximum germination (higher asymptote),  $t$  is the time (in days),  $a$  represents the slope of the curve, and  $b$  the inflexion point.

The goodness-of-fit was evaluated with the model efficiency index (EF; Loague & Green, 1991). The model EF was calculated as follows:

$$EF = \frac{\sum(O_i - \bar{O})^2 - \sum(P_i - O_i)^2}{\sum(O_i - \bar{O})^2} \quad (2)$$

where  $P_i$  is the predicted value,  $O_i$  is the observed value, and  $\bar{O}$  is the mean of the observed values. The value of EF can range from  $-\infty$  to 1. For an ideal fit, the EF value equals 1.

The times necessary for the germination of half of final germinated seeds ( $t_{50}$ ), which in the above equation correspond to the inflexion points ( $b$ ), were considered statistically different (0.05 probability level) according to the criterion that their respective 95% confidence intervals did not overlap, as already adopted in other studies (Loddo et al., 2012, 2013).

#### *Final germination percentage*

Data of final germination percentage (number of germinated seeds on total sown seeds per sample) were transformed by the arcsine of square root transformation to reduce non-normality of the dataset distribution. This result was confirmed by analysing plots of residuals. ANOVA was performed on transformed data using the general linear models module of STATISTICA 7.1 (StatSoft Inc., 2005). Graphs and discussion are based on untransformed data. Fisher’s protected least significant difference test was used at the 0.05 level of probability to identify significant differences between means.

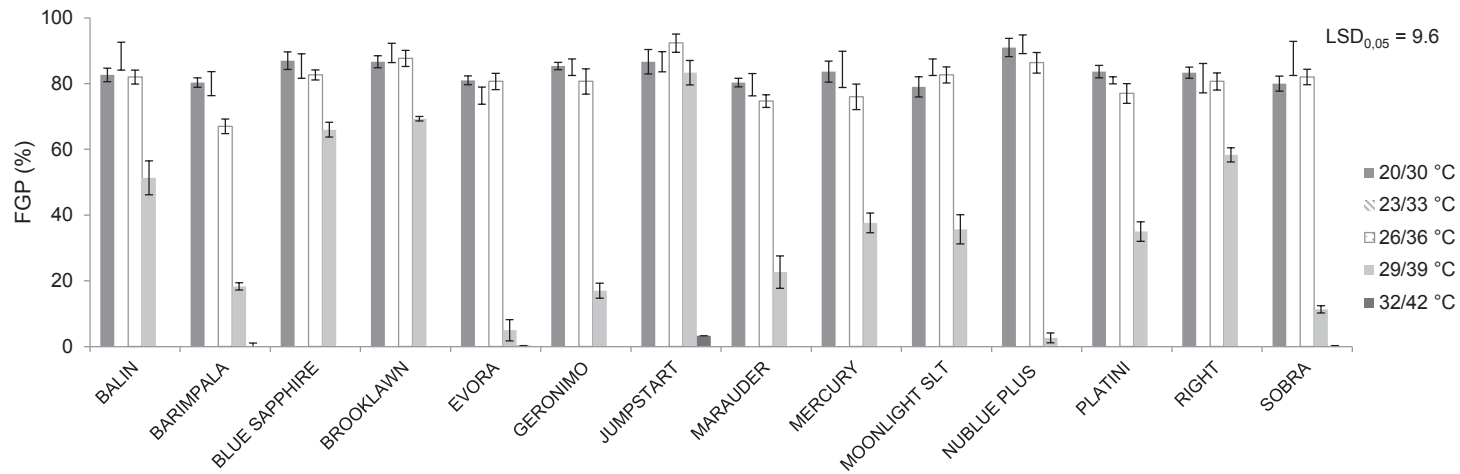
## RESULTS AND DISCUSSION

At alternating temperature of 32/42 °C none of the tested cultivars germinated, with the exception of very few seeds of 'Jumpstart' and 'Evora' (Fig. 1). For all the other cultivars, the 29/39 °C treatment was the highest temperature regime where germination was observed. Germination was high under temperature regimes of 20/30, 23/33 and 26/36 °C, and differences among cultivars were limited. At regime 29/39 °C the cultivars clearly displayed the greatest difference in their ability to germinate, 'Jumpstart' showed the highest final germination percentage, while 'Evora' and 'Nublue Plus' had the lowest (Fig. 1). At 29/39 °C only five cultivars reached at least 50% of germination ('Balin', 'Blue Sapphire', 'Brooklawn', 'Jumpstart' and 'Right'). This result corroborates those of Van't Klooster (2007) who observed distinct temperature regimes for germination in various cultivars of Kentucky bluegrass and good germination in some of them at high temperatures (up to 35 °C).

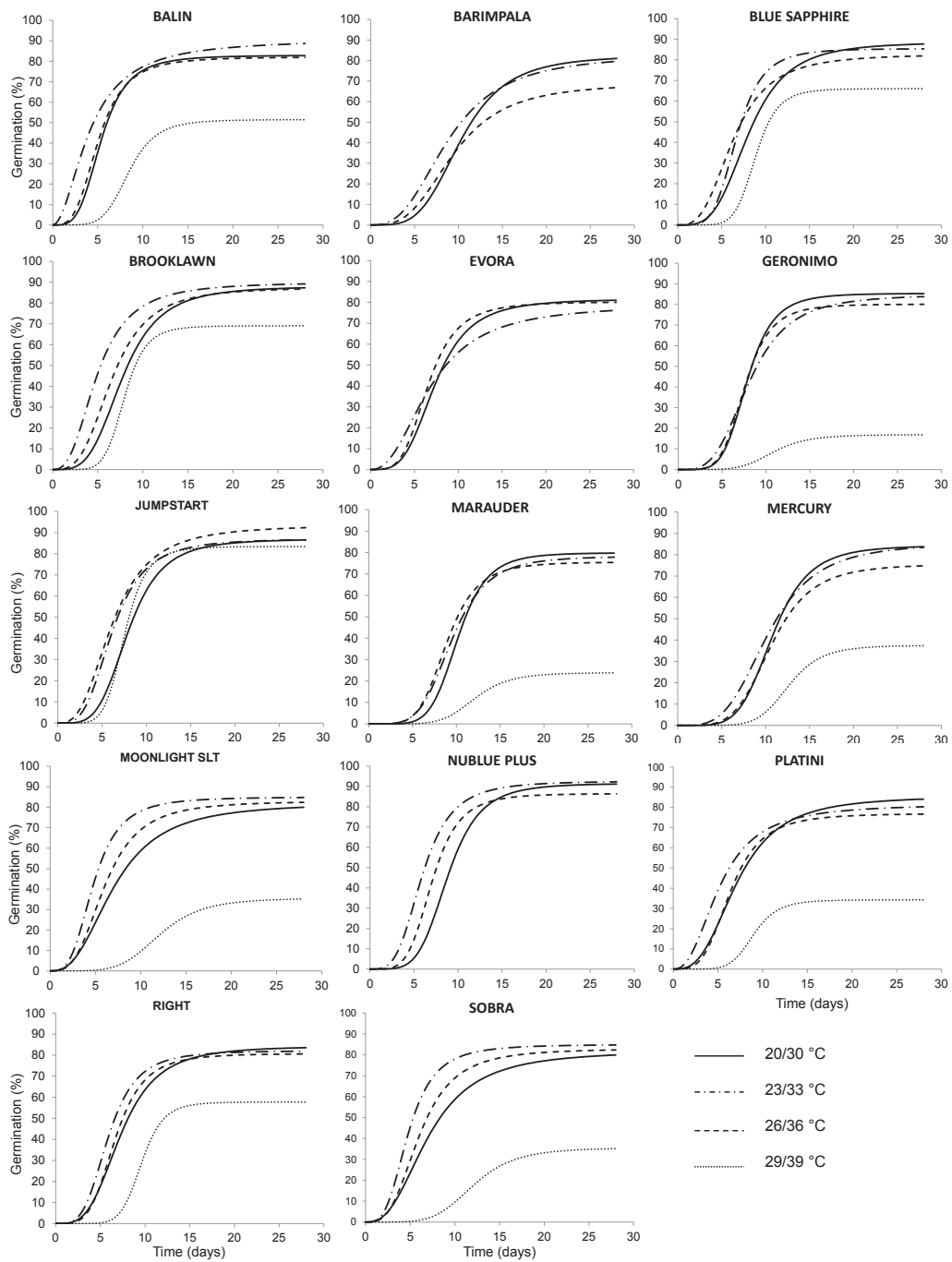
The germination patterns were also similar between cultivars under temperature regimes of 20/30, 23/33, and 26/36 °C (Fig. 2), as confirmed comparing their  $t_{50}$  (if their respective 95% confidence intervals do not overlap, they are considered statistically different at  $P < 0.05$ ) (Table 1). The statistically significantly  $t_{50}$  was reached later than at the other temperature regimes (95% confidence intervals do not overlap) only under 29/39 °C by the cultivars 'Moonlight Slr' and 'Sobra'. The  $t_{50}$  ranged between 4 (at 23/33 °C) and 12 days (at 29/39 °C). For the cultivars 'Barimpala', 'Evora' and 'Nublue Plus' at 29/39 °C there were too few data points for an acceptable non-linear regression, therefore no  $t_{50}$  was estimated. EF of the logistic functions were in almost all cases more than 0.9, with just a few exceptions at 29/39 °C; in any case the fitting was satisfactory considering that an EF of 0.5 is suggested as the lower range value for acceptable model prediction (Ramanarayanan et al., 1997).

The different responses observed at the temperature regime of 29/39 °C indicate a real risk of poor germination for several cultivars in the case of spring sowing. A maximum temperature of 39 °C or more in the soil germination layer (0–5 cm) can be expected in temperate climate zones, such as northern Italy, in late spring (June) (Loddo et al., 2015). Due to global changes in climate that are expected to affect not only average, but also extreme temperatures, this critical temperature is also predicted to occur earlier, in early spring. Therefore, in the future, when sowing is planned in either early or late spring, it is reasonable to select cultivars with high germination capacity under high temperatures to avoid possible turf establishment failure.

In the coming years, cultivar selection based on germination rate at high temperatures could be essential to obtain a rapid and regular establishment of Kentucky bluegrass turfgrasses. A sparse stand derived from poor germination due to heat damage has a direct negative influence on turf quality and wear tolerance (Fiorio et al., 2012). Moreover, poor density in the initial phase of establishment may have a negative environmental impact because it leaves gaps for a weed infestation that requires treatment with chemicals.



**Figure 1.** Final germination percentage (FGP) after 28 days in a conditioning chamber under five regimes of alternating temperatures (20/30–32/42 °C) of fourteen Kentucky bluegrass cultivars (bars represent standard error of the mean, LSD value at 0.05 level of significance is reported in figure).



**Figure 2.** Germination pattern of fourteen Kentucky bluegrass cultivars at four regimes of alternating temperatures (20/30–29/39 °C).

**Table 1.** Time to reach 50% of germination and efficiency index of the logistic functions for each Kentucky bluegrass cultivar and each alternating temperature regime

Cultivar	Temp. regime (°C)	t <sub>50</sub> (days)	St. err.	Upper limit (0.95)	Lower limit (0.95)	EF
Balin	20/30	5.39	0.516	6.43	4.35	0.98
	23/33	4.07	1.606	7.37	0.78	0.93
	26/36	5.06	1.150	7.42	2.70	0.96
	29/39	8.44	0.594	9.66	7.22	0.94
Barimpala	20/30	10.28	0.425	11.14	9.42	0.96
	23/33	8.85	0.555	9.99	7.71	0.96
	26/36	9.44	0.460	10.38	8.49	0.97
	29/39	--	--	--	--	--
Blue Sapphire	20/30	8.08	0.165	8.41	7.75	0.99
	23/33	6.73	0.172	7.08	6.37	0.98
	26/36	6.33	0.293	6.93	5.73	0.97
	29/39	8.86	0.598	10.08	7.63	0.97
Brooklawn	20/30	7.73	0.174	8.09	7.38	0.99
	23/33	4.90	0.570	6.07	3.73	0.98
	26/36	6.59	0.311	7.23	5.96	0.96
	29/39	7.96	0.508	9.00	6.91	0.97
Evora	20/30	7.36	0.229	7.83	6.90	0.97
	23/33	6.82	0.918	8.70	4.94	0.86
	26/36	6.55	0.232	7.03	6.08	0.97
	29/39	--	--	--	--	--
Geronimo	20/30	7.84	0.168	8.18	7.50	0.99
	23/33	8.09	0.651	9.43	6.76	0.91
	26/36	7.58	0.297	8.19	6.97	0.97
	29/39	10.85	1.592	14.12	7.59	0.73
Jumpstart	20/30	7.97	0.184	8.34	7.59	0.99
	23/33	6.17	0.393	6.98	5.37	0.95
	26/36	6.14	0.240	6.64	5.65	0.98
	29/39	7.53	0.188	7.92	7.15	0.99
Marauder	20/30	10.16	0.526	11.23	9.10	0.95
	23/33	9.48	0.533	10.57	8.39	0.95
	26/36	8.89	0.371	9.66	8.13	0.97
	29/39	12.14	1.699	15.63	8.66	0.72
Mercury	20/30	10.81	0.338	11.49	10.13	0.98
	23/33	10.28	0.471	11.25	9.32	0.97
	26/36	10.69	0.489	11.69	9.69	0.96
	29/39	12.67	0.505	13.71	11.63	0.97
Moonlight slt	20/30	7.05	0.409	7.88	6.23	0.95
	23/33	4.79	1.402	7.67	1.92	0.96
	26/36	6.04	0.257	6.57	5.51	0.98
	29/39	12.13	0.518	13.19	11.07	0.96
Nubblue plus	20/30	8.84	0.201	9.24	8.43	0.99
	23/33	5.95	0.350	6.67	5.23	0.98
	26/36	7.06	0.161	7.39	6.74	0.98
	29/39	--	--	--	--	--

Table 1 (continued)

Platini	20/30	7.13	0.176	7.49	6.77	0.99
	23/33	5.19	0.626	6.48	3.91	0.96
	26/36	6.41	0.237	6.90	5.93	0.98
	29/39	9.00	1.594	12.27	5.73	0.77
Right	20/30	7.27	0.238	7.75	6.79	0.97
	23/33	5.88	0.669	7.26	4.51	0.94
	26/36	6.68	0.219	7.13	6.23	0.97
	29/39	9.61	1.191	12.05	7.17	0.90
Sobra	20/30	7.05	0.409	7.88	6.23	0.95
	23/33	4.79	1.402	7.67	1.92	0.96
	26/36	6.04	0.257	6.57	5.51	0.98
	29/39	12.13	0.518	13.19	11.07	0.96

$t_{50}$  – time to reach 50% of germination; St.err – standard error of  $t_{50}$ ; Upper and Lower limits (0.95) – 95% confidence limits of  $t_{50}$ ; EF – efficiency index.

## CONCLUSIONS

The results of this study demonstrated good germination of all the Kentucky bluegrass cultivars at the three lower temperature regimes tested (20/30, 23/33, 26/36 °C). There were large and significant differences among cultivars at 29/39 °C with only five reaching almost 50% of germination. At this temperature regime ‘Jumpstart’ showed the highest germination percentage (83%). Results suggest that breeding for traits such as germination at high temperatures may help develop cultivars that can be sown successfully in the spring. Improved cultivars with these traits will represent an excellent choice for transition zone areas, such as northern Italy, where extreme weather events may become more common as a result of climate change. This study focused on germination, which is certainly a key factor, but it is only a part of the establishment phase that is very critical for growing high-quality durable turfgrass. These results should therefore be validated through a field study not only to compare laboratory tests with field conditions but also to understand the influence of germination temperature response on speed of establishment.

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