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DIFFERENCES OF THE BOG AND DRY SITE SCOTS PINE POPULATION SEEDLINGS GERMINATION AND EARLY GROWTH

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Scots pine is wind dispersed species; both seeds and pollen are dispersed by wind. Germination is very important process which depends on different biotic and abiotic factors and if conditions are inappropriate (wrong time, location) then this process causes death of the individual and may have an important role for the population. Scots pine germination under field conditions depends on soil temperature, moisture, light intensity, seed color, mass, seed source. On natural conditions germination process depends on microhabitats, too. Bogs are ombrotrophic peatlands with a surplus of water (usually) and more than 30 cm of peat layer. This means that Scots pine has to adapt to survive in harsh conditions. The main objective of this research is to identify if early growth and development of Scots pine seedlings from bog site differs from seedlings from dry sites if growing in the same conditions. Differences between bog site and dry site seeds mass and germination are statistically significant, but germination energy does not differ between sites. There are no statistically significant differences between bog site and dry site seedlings at the beginning of the early growth. Bog site and dry site seeds germination energy is quite similar and it means that on harsh (natural) conditions seeds have equal possibilities to germinate. During the first two weeks seedlings from both sites develops equally and only latter differences in early growth become visible and meaningful.

Key words: bog, germination, Scots pine, seedling, terminal bud.

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INTRODUCTION

Germination is very important process which depends on different biotic and abiotic factors and if conditions are inappropriate (wrong time, location) then this process causes death of the individual and may have an important role for the population. Scots pine germination under field conditions depends on soil temperature, moisture and light intensity (Castro et al. 2005). If it is rainy season germination reach high percentage in all microhabitats (even understory, too). In contrast, during the dry season the highest germination rate is in those microhabitats which reach higher soil temperature (sunny ones). Different microhabitats guaranty good germination conditions during different seasons (Oleskog & Sahlén 2000).

On natural conditions germination process depends on microhabitats. Bogs are ombrotrophic peatlands with the surface above the surrounding terrain or otherwise isolated from laterally moving mineral-rich soil waters and peat layer is almost always more than 30 cm deep (Rydin et al. 2013). Development of Scots pine in bogs is mainly determined by local factors (Dauškane et al. 2011, Hökkä et al. 2012) and is largely controlled by site-specific, very local conditions (Ohlson 1995, Leishman & Westoby 1994).

The germination in peatland is high, 76% on hummocks and 66% in hollows, but it is not significantly different between the microhabitats (Gunnarsson & Rydin 1998). Up to 75% average germination is reported from sowing experiments in boreal bogs (Gunnarsson & Rydin 1998), while germination typically averages - 5% in boreal forests (Zackrisson et al. 1997). *Sphagnum angustifolium* microhabitats is most favorable for germination of Scots pine but mortality rate is highest, too (Ohlson & Zackrisson 1992).

Pinus sylvestris seedlings have the capability to germinate and establish on the central bog if they have a sufficient time of favorably unwaterlogged conditions in the first phases of germination (Mukasabi et al. 2012). Early seedling survival is the most severe bottleneck for tree recruitment in bogs because more than 90% of all germinated seedlings do not survive (Ohlson et al. 2001).

The main objective of this research is to identify if early growth and development of Scots pine seedlings from bog site differs from seedlings from dry sites if growing in the same conditions. In order to reach this goal, the main objectives were held:
1. To compare Scots pine seeds from bog site and dry site germination;
2. To compare Scots pine seedlings from bog and dry sites development;
3. To compare Scots pine seedlings from bog site and dry site the end of the first vegetations period.

MATERIAL AND METHODS

Scots pine cones were collected at spring time. 20 trees in dry site and 20 trees in bog site were selected randomly. 20 cones from each tree were taken (in total 400 cones from dry site and 400 cones from bog site).

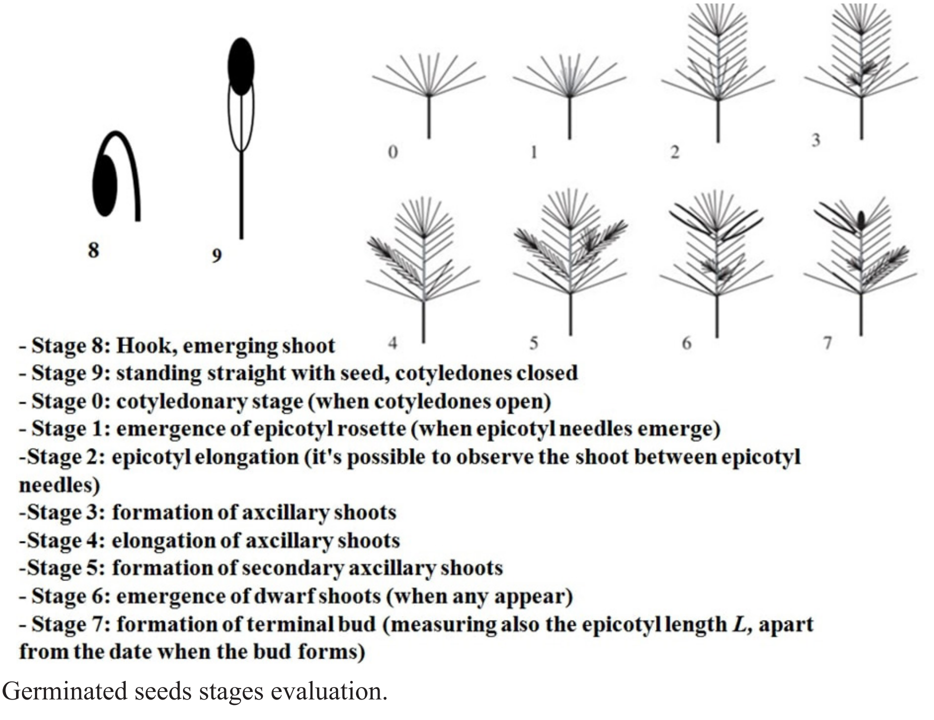

Fig. 1 Germinated seeds stages evaluation.

According to Aniszewska (2013) two stages seeds extraction method seeds were extracted. Cones dried in oven Triter kb-8182 at 35˚C and 50˚C temperature. Seeds from different trees were extracted manually and kept separately. Extracted seeds were stored in 0-4 ˚C temperature till sowing time.

For the sowing experiment 80 full (vigor) seeds from each tree were selected and sown in special pots according to the scheme. In total 1600 seeds from dry site and 1600 seeds from bog site were sown. Sown experiment was held in a greenhouse for 5 month (June-October).

Seeds mass was calculated before sowing. Seeds were weighted with PS 600/C/2. Four times 100 seeds were weighted and then the average of 100 seeds was calculated. After that mass of 1000 seeds was calculated.

A seed is considered to have germinated after the emergence and development from the seed embryo of those essential structures which are indicative of the seed's capacity to produce a normal seedling under favorable conditions. If the sprout is two times greater than the seed – it was counted as germinated.

Germination energy for the Scots pine is evaluated seven days after sowing – germinated seeds number is compared with total sown seeds number. Germination for the Scots pine is evaluated 21 day after sowing – germinated seeds number is compared with total sown seeds number. Germination energy and germination are counted in percentage.

We classified germinated seeds/seedlings development into seven stages (Fig. 1).

If the sprout is two times bigger than the seed – it is counted as germinated (stage 8 – hook) and after that the development of the sprout is classified into stages from the cotyledons closed to the formation of terminal bud.

RESULTS AND DISCUSSION

1600 seeds from the dry site and 1220 seeds from the bog site were sown. From the sowing date till August 17'th the number of seedlings from the dry site was growing (Fig. 2): from 1180 on germination counting date (June 25'th) till 1206 on August 17'th. Number of seedlings from the bog site has the same tendency – it is growing: starts with 586 seedlings on germination counting date (June 25'th) and grows till 664 on July 28'th. In both cases, later on the number of seedlings decreases.

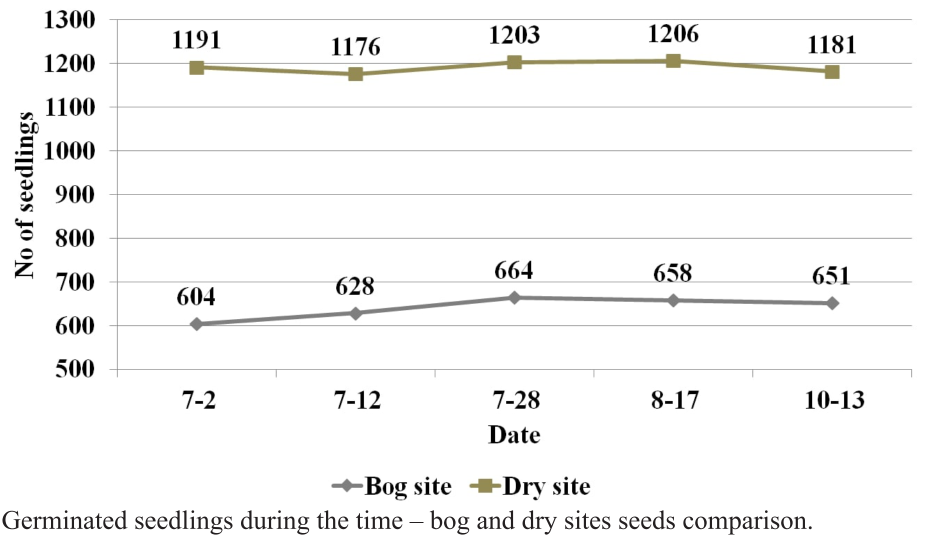

Fig. 2 Germinated seedlings during the time – bog and dry sites seeds comparison.

Table 1. T-test results; pink – statistically significant

t-Test: Paired Two Sample for Means

	B1000	*J1000*	*GermB*	*GermJ*	*GermEB*	*GermEJ*
Mean	3.54	8.24	48.40	73.31	5.25	3.75
Variance	0.61	2.12	645.86	193.80	36.72	23.36
Observations	20	20	20	20	20	20
Pearson Correlation	-0.38		0.16		-0.16	
Hypothesized Mean Difference	0		0		0	
df	19		19		19	
t Stat	-11.0857		-4.1309		0.8043	
P(T<=t) one-tail	**0.0000**		**0.0003**		**0.2156**	
t Critical one-tail	1.7291		1.7291		1.7291	
P(T<=t) two-tail	**0.0000**		**0.0006**		**0.4312**	
t Critical two-tail	2.0930		2.0930		2.0930	

During all period the number of dry site seedlings is 1.8-1.9 time greater than the number of seedlings from the bog site. Germinated and growing seedlings from the dry site consist about 74 % of all sown seeds and from the bog site – about 53 % .

Bog site seed mass vary from 1.32 g till 4.92 g and the mean of 1000 seeds mass is 3.54 g (Fig. 3). 1000 seeds from the dry site mass vary from 6.20 g till 11.20 g and the mean of 1000 seeds is 8.24 g (Fig. 3). Seeds from the dry site are 2.3 times heavier. Seed mass in Scots pine is strongly influenced by the genetic constitution of the maternal parent and is highly constant within maternal parents (Castro 1999). The early growth is largely determined by seed mass (Reich et al. 1994), however, after one growing season, seed mass had no effect on seedling performance, which depended exclusively on maternal origin (Castro 1999).

Mean germination energy in the bog site is 5.25 % and in the dry site – 3.7% (Fig 3). Seeds from the bog site have higher germination energy. Mean germination in the bog site is smaller than in the dry site – 48.4 % and 73.31 % respectively (Fig 3). Scots pine seeds do not normally have any dormancy and have the ability to germinate immediately upon releases from the cone (Castro et al. 2005). Up to 75% average germination is reported from sowing experiments in boreal bogs (Gunnarsson and Rydin 1998), while germination typically averages - 5% in boreal forests (Zackrisson et al. 1997).

In order to compare bog and dry sites seeds mass, germination energy and germination – t-test was selected. According to the results – there are statistically significant differences between 1000 seeds mass and germination, but germination energy do not differ between sites – difference is not statistically significant (Table 1).

Different researches show different result in seed mass effect on germination. In our research regression analysis reveals weak relationship between 1000 seeds mass and germination in both cases – dry site and bog site seeds (Fig. 4). Coefficient of determination show that only 26 % of all variation in linear model is explained by the 1000 seeds mass in dry site and 18 % in bog site. It is generally accepted that larger seeds give rise to seedlings with better performance (Castro 1999). On the other hand, the size that a seed reaches is genetically determined by at least two different traits; the genetic variability of the developing embryo and the genetic variability of the maternal plant (Castro 1999). Seed mass is important factor for seedlings mass only during the first growth year (Reich et al. 1994).

Early growth stages changes thru the time – since the emergency from the soil (stage 8) till the terminal bud formation (stage 7). Terminal bud might be formed during different early growth stages – seedling during the first growing season formed terminal bud on stage 2 till stage 6. Seeds from the bog site have a smaller seed mass then the seeds from the dry site. It is proved that early growth of seedlings is determined by the seed mass (Reich et al. 1994).

In comparison, seedlings of stage 0 in bog site there is 12 % at the beginning of the experiment (July 2'nd) and this share decrease during the first month till 1.8 % on July 28'th. The same tendency is on dry site just this process is faster – seedlings of stage 0 consist 5.7 % at the beginning (July 2'nd) and 0.8 % at the end of first month (July 28'th) (Figs 5, 6).

On both cases during the first half of July seedlings do not reach the stage 2. This stage is captured only on July 28'th and consist 85 % in the bog site and 92 % in the dry site. Stage 3 is also captured in this time and is more common in bog site – 5 % from all seedlings (1.8 % in dry site seedlings). At the end of the experiment seedlings of stage 3 consist about 34 % in both cases. On this stage formation of axcillary shoots starts. The initial growth of the shoot is positively correlated with seed mass (Castro 1999). In our research the bog site seeds have smaller mass then the dry site seeds, so according the previous statement, dry site seedling should have higher rate of stage 3. But in contrary, bog site seedlings started to form axcillary shoots first. Of course, at the end of the experiment, the rate of stage 3 in both cases was similar. Seed mass effect on different traits is variable. In our case, seeds origin from very different habitats should have an effect,

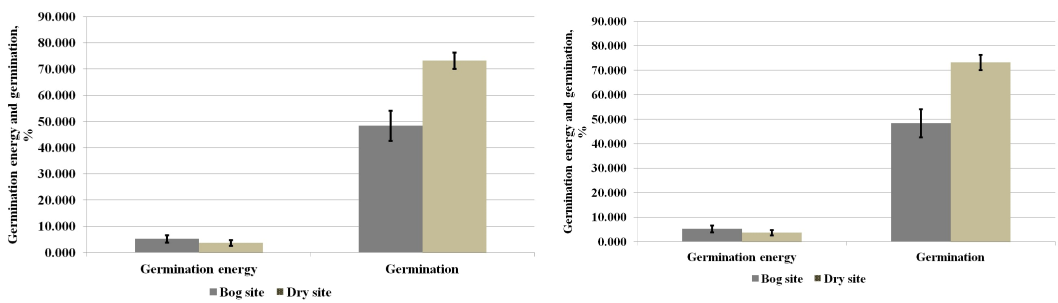

Fig. 3 Mean germination energy (%), mean germination (%) and mean 1000 seeds mass of bog site and dry site seeds; standard error.

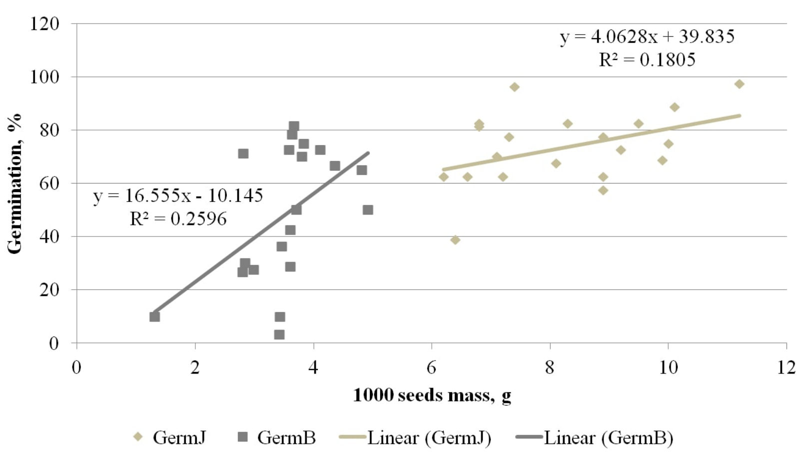

Fig. 4 Germination dependence on 1000 seed mass in dry site and bog site seedlings.

not just seeds mass, maternal parents, soil type or favorable conditions in nursery.

Stage 4 occurred on August 17'th in dry site and bog site seedlings and consist 2.5 % and 0.9 % respectively. On October 13'th 5.6 % of dry site seedlings and 7 % of bog site seedlings were in stage 4. Stage 6 is more common for bog site seedlings and occurred at the end of vegetation. 1.5 % of bog site seedlings were in this stage on October 13'th. Only 0.08 % of dry site seedlings had this stage.

According to the Ohlson (1999) research, the traits on mineral soil are very similar in the bog and neighboring mineral soil pine populations. In contrast, if growing on the bog the native bog pines grow faster and have a larger proportion root than those originating from the population on the adjacent mineral soil (Ohlson 1999). Soil type influenced Scots pine seedlings emergence and survival (Castro 1999).

ANOVA analysis comparing bog site and dry site seedlings early development stages results show, that differences' between two groups are

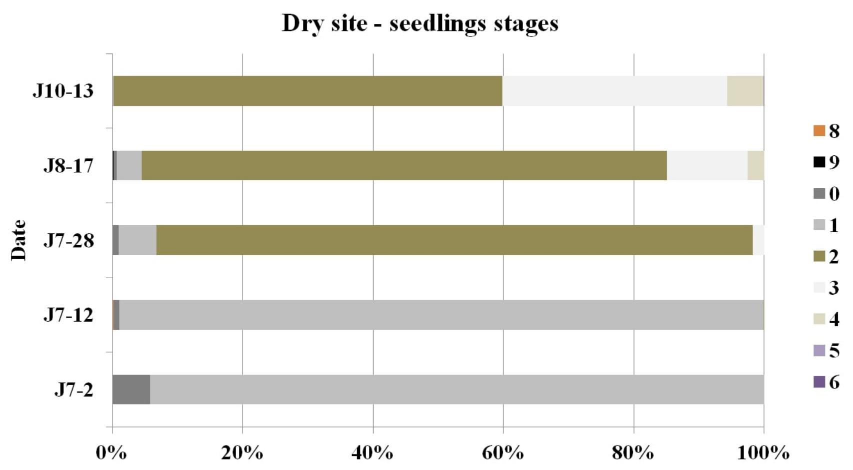

Fig. 5. Dry site seedlings early growth stages change during the first season till terminal bud formation.

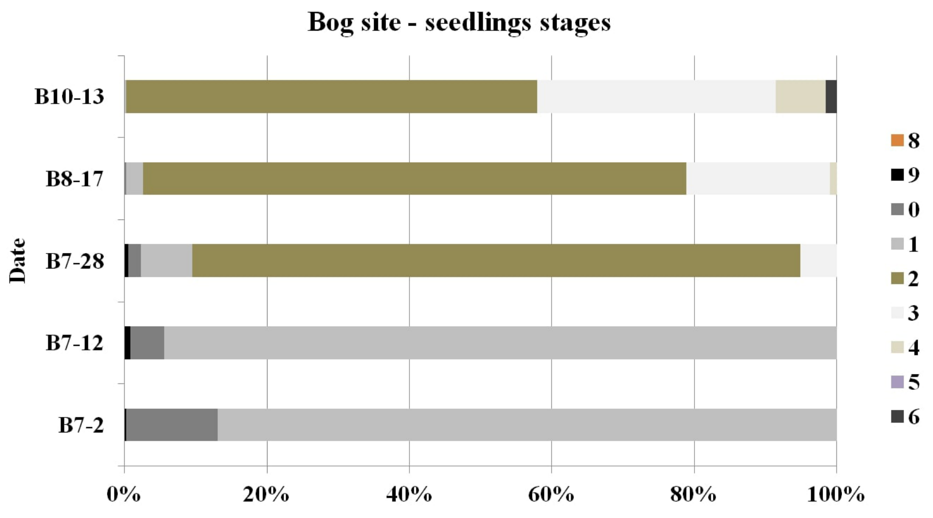

Fig. 6. Bog site seedlings early growth stages change during the first season till terminal bud formation.

Table 2. Bog site and dry site seedlings development stages differences. Tukey's Q below the diagonal, p (same) above the diagonal. Significant comparisons are pink

Date	**07-02**	**07-12**	**07-28**	**08-17**	**10-13**
07-02		0.9297	0.0172	0.0172	0.0172
07-12	1.14		0.0172	0.0172	0.0172
07-28	77.00	78.14		0.0172	0.0172
08-17	91.51	92.64	14.51		0.0172
10-13	115.50	116.60	38.50	23.99	

Table 3. Two-way ANOVA results for bud formation in bog site and dry site seedlings and Tukey's post-hoc for interaction between two nominal variables

	Sum of sqrs	df	Mean square	F	p (same)
Bud-7,no bud-1:	57.2230	1	57	12.87	0.0003
Bog-1,dry-2:	24.3910	1	24	5	0.0193
Interaction:	814.0770	1	814	18.31	0.0198
Within:	772.9920	1739	0.4445		
Total:	789.5940	1742			

Tukey's post-hoc-INTERACTION						
		Q	p			
7-1	7-2	7.2120	0.0226		7	Bud
7-1	11-1	8.3160	0.0218		11	No bud
7-2	11-2	0.7421	0.8592		1	Bog site
11-1	11-2	0.4546	0.9446		2	Dry site

statistically significant ($p = 0.000$). In order to find which differences are statistically significant Tukeys's test was done (Table 2).

Tukey's test results show that there are no statistically significant differences between bog site and dry site seedlings at the beginning of the experiment (Table 2) but latter on all comparisons are significant. Bog site and dry site seeds germination energy is quite similar and it means that on harsh (natural) conditions seeds have equal possibilities to germinate. During the first two weeks seedlings from both sites develops equally and only latter differences in early growth become visible and meaningful.

Terminal bud formation means the end of vegetation season. In both cases seedlings with formed terminal bud consist about 30 % at the end of the experiment (October 13'th).

In order to analyze if there are differences between bog site and dry site seedlings terminal bud formation two-way-ANOVA test is done (Table 3). We analyze two null hypothesis: fist of all – development stages of seedlings are equal between those seedlings which formed terminal bud and those which do not; secondly, development stages are equal between bog site seedlings and dry site seedling at the end of the experiment (October 13'th). In addition to this, there is no interaction between terminal bud formation and seedlings site. According to the results of two-way ANOVA test interaction term is significant ($p = 0.02$). That means – the effect of one factor (bud formation) depends on the other factor (seedlings site) (Table 3 – Tukey's post-hoc).

For bog site seedlings stage 6 (emergency of dwarf shoots) and stage 4 (elongation of axcillary shoots) are more common at the end of the experiment so mean development stage has a higher value than the dry site seedlings (Fig. 7). In addition to this, tendency that terminal bud of bog site seedlings is more often formed at stage 6 or stage 4 also is clear. Contrary, dry site seedlings do not have such evident differences between stages when terminal bud is formed.

Seeds from the dry site and seeds from the bog site have very similar germination energy but germination of those two sites seeds differs significantly. Influence of seed mass on germination meets the results of other researchers – there is a week relationship between these two traits. Early growth seedling stages changes faster in dry site seedlings. Bog site seedlings have higher rate of stage 6 – formations of dwarf shoots. Terminal bud formation depends on seeds site (bog or dry) and there are significant differences between terminal bud formation on bog site and dry site seedlings.

CONCLUSION

Differences between bog site and dry site seeds mass and germination are statistically significant, but germination energy does not differ between sites.

There are no statistically significant differences between bog site and dry site seedlings at the beginning of the early growth but latter on all comparisons are significant. Bog site and dry site seeds germination energy is quite similar and it means that on harsh (natural) conditions seeds have equal possibilities to germinate. During the first two weeks seedlings from both sites develops equally and only latter differences in early growth become visible and meaningful.

Bog site and dry site seedlings with formed terminal bud consist about 30 % at the end of the experiment. For bog site seedlings stage 6 (emergency of dwarf shoots) and stage 4 (elongation of axcillary shoots) are more common at the end of the experiment so mean development stage has a higher value than the dry site seedlings. In addition to this, tendency that terminal bud of bog site seedlings is more often formed at stage 6 or stage 4 also is clear.

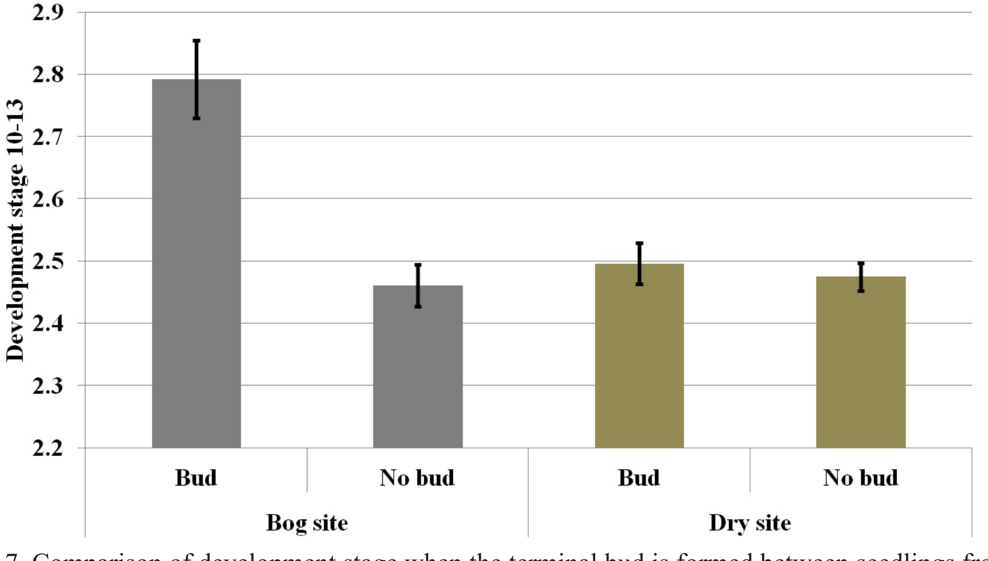

Fig. 7. Comparison of development stage when the terminal bud is formed between seedlings from bog site and dry site (mean development stage and standard error).

Contrary, dry site seedlings do not have such evident differences between stages when terminal bud is formed.

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