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Germinative capacity and energy of critically endangered Ojców birch (*Betula oycoviensis* Besser) in the Czech Republic

Josef Gallo*, Zdeněk Vacek, Martin Baláš, Stanislav Vacek

Department of Silviculture, Faculty of Forestry and Wood Sciences, Czech University of Life Sciences Prague, Prague, Czech Republic

**Corresponding author: gallo@fld.czu.cz*

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Abstract: Ojców birch (*Betula oycoviensis* Besser) is a rare and critically endangered taxon of the genus *Betula*. Its distribution is limited to few countries in Europe. In the Czech Republic, this taxon, characterized by typical shrubby habitus, has been found in fewer than 70 tree individuals, prevailingly in the studied locality Volyně, West Bohemia. This study was focused on the germinative capacity and germinative energy of this taxon, as it is an important indicator of possible regeneration of the trees in nature and for conservation. Non-stratified seeds (2 200 pcs) and stratified ones (2 200 pcs) were compared to each other and in relation to dendrometric tree parameters. The results showed no significant ($P > 0.1$) differences between stratification variants in total germination (higher by 0.8% in stratified), though stratification improved germinative energy. Germination was in 1st week higher by 6.0% in stratified variant, respectively marginally ($P < 0.1$) higher in 2nd week in non-stratified variant. However, both germinative capacity and germinative energy were significantly ($P < 0.01$) variable between individual trees. The germinative capacity was significantly ($P < 0.05$) positively correlated with tree defoliation. Tiny seeds and triploid trees exhibited very low and zero germinative capacity and energy, respectively. Totally, seeds exhibited sufficient germinative capacity and germinative energy of 23.1% (0.0–62.5%). This suggests that the trees can be potentially used for this type of Ojców birch regeneration i.e. generatively from seeds, although rather in controlled artificial conditions.

Keywords: germination; stratification; silviculture; Ore Mts.; Central Europe

Birches (*Betula* L.) are an essential ecological component in European forests (Hynynen et al. 2010; Vacek et al. 2016). This genus plays an important role in the Czech Republic in terms of increasing biodiversity, complex forest structure and improving soil properties (Podrázský et al. 2009). Birches are light-demanding pioneer tree species, which rapidly occupy open areas after forest fires, post-mining landscape and clear-cuttings due to their prolific seed production and fast juvenile growth (Fischer et al. 2002; Vacek et al. 2018a). On the other hand, birch may have commercial importance, especially silver birch (*Betula pendula* Roth) and downy birch (*Betula pubescens* Ehrh.) (Valkonen, Valsta 2001; Hynynen et al. 2010).

In Czech Republic, rare taxon of the genus *Betula* Ojców birch (*Betula oycoviensis* Besser) occurs in addition to the two species mentioned above (Karlík et al. 2010; Baláš et al. 2016). Its distribution is limited to several countries in Central, Northern and Eastern Europe (Staszkiewicz 2013; Vítámvás et al. 2020). At the study area Volyně, Ojców birch

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also suffers from high intensity of deer browsing, such as other palatable broadleaved tree species (Vacek et al. 2013; Ambrož et al. 2015). Therefore, the ability (density and growth) of natural regeneration and occurrence of young trees is considerably decreased due to increasing deer population in the Czech Republic (Vacek 2017; Cukor et al. 2019). Moreover, regeneration of Ojców birch is strictly limited on study site due to dense grass sward (Baláš et al. 2016). Climatic extremes in recent years could also contribute to less vigorous growth and regeneration of birch, as it is case of many other tree taxons (Leslie et al. 2017; Vacek et al. 2017b; Gallo et al. 2020). On the other hand, birch (especially Carpathian birch [*Betula carpatica* W. et K.]) is relatively vulnerable to climatic extremes and air pollution load (Balcar, Kacálek 2001; Novák et al. 2017), that these stresses historically caused decline and large-scale disturbance of other tree species in study mountain range systems (Král et al. 2015; Vacek et al. 2017a; Putalová et al. 2019).

For silviculture and forest management, it is particularly important to differentiate between *Betula pendula* Roth group and *B. pubescens* group and select them into mixtures according to site specifications to ensure best possible vitality of the stands in future (Linda et al. 2016). B. *pubescens* (unlike *B. pendula*) can be successfully in terms of silviculture in mixtures with, for example, spruce, as it does not damage spruce crowns by whipping (Poleno et al. 2009). In this role, *B. oycoviensis* belongs to the *B. pendula* Roth and usually it is not differentiated (Kuneš et al. 2019). This critically endangered taxon was first described in 1805 (Besser 1809). Ojców birch has prevailingly a shrubby habitus of height around 4–7 m, but on some locations it reaches dimensions of medium-size trees with maximum height up to 20 m with typical morphology, such as curved and dense branching, dormant buds and epicormic shoots (Staszkiewicz, Wójcicki 1992; Baláš et al. 2016). It is evident that there is no potential commercial use of Ojców birch in forestry, however different growing forms of same species increase the genetic biodiversity of forests (Ivetić et al. 2016).

Successful generative reproduction and following natural or artificial regeneration is one of key factors for successful forest management and silvicultural practise (Karlsson 2001; Vacek et al. 2017c), such as in given species. Ojców birch is a rare and declining tree taxon, which is without active management likely to disappear from its important locations of occurrence. Seed production and subsequent germination play an important role for successful birch reproduction in terms of both natural and artificial regeneration (Hester et al. 1991; Cameron 1996). Moreover, stratification can improve the germinative capacity of this tree species (Ahola, Leinonen 1999; Mir et al. 2018). For these reasons, presented study is focused on the germination of this rare taxon, as it is an important indicator of possible generative regeneration of the Ojców birch in nature and for conservation. Moreover, data and research dealing with germination of Ojców birch are missing. The objectives of this research were to (1) determine germinative capacity and germinative energy, (2) compare difference in germination of non-stratified and stratified seeds and (3) observe relationship germination, tree parameters and health status of this rare critically endangered taxon.

MATERIAL AND METHODS

Study area. The seed material of Ojców birch was collected in Volyně settlement near Chomutov, West Bohemia, Czech Republic. The locality is situated in the Ore Mts. of altitude range 711–745 m a.s.l. on nutrient-poor abandoned pastures. Area of interest is located close to Natural Monument Locality Ojców birch near Volyně that was established in 1986 of size 1.03 ha due to occurrence of critically endangered Ojców birch. Soil type is dystric cambisol and prevailing bedrock is schist and gneiss. Mean annual air temperature reaches 6.5 °C and annual sum of precipitation is 700 mm. The study territory belongs to humid continental climate characterized by hot and humid summers and cold to severely cold winters (Dfb) according to Köppen climate classification (Köppen 1936), respectively by detailed region Quitt distribution (Quitt 1971) to a cold climatic region and CH 7 subregion.

Data collection. Seeds were collected in September after seed year 2018 from morphologically typical Ojców birches and from those that carried sufficient number of full seeds. Seeds were taken at tree height 1–4 m from at least 3 whorls and 2 branches. Dendrometric parameters and health status (foliation) of sampled trees were measured (Table 1). Diameter at breast height (DBH) was measured by Blue Mantax caliper (Haglöf, Sweden) to an accuracy of 1 mm. Tree height, base of live crown and

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Table 1. Characteristics of trees sampled for seed material in the locality Volyně

Tree ID*	GPS		Altitude (m a.s.l.)	Height (m)	DBH (cm)	SQ	Crown width (cm)	Crown base (cm)	Foliation (%)
	Northing	Easting							
VOL-24D	50.44233	13.21838	713	5.0	11.5	43.5	3.0	2.5	80
VOL-16D	50.44249	13.21829	716	4.6	8.0	57.5	2.2	1.9	40
VOL-06D	50.44553	13.20653	718	2.3	3.8	60.5	2.3	1.5	55
VOL-03D	50.44485	13.21222	745	11.8	12.4	95.2	4.1	6.5	50
VOL-15D	50.44263	13.21842	717	11.5	10.8	106.5	4.8	6.0	55
VOL-25D	50.44235	13.21862	711	2.5	4.5	55.6	1.5	1.3	35
VOL-30D	50.44251	13.21845	715	6.4	9.2	69.6	2.5	2.2	40
VOL-32D	50.44285	13.21868	714	4.4	6.0	73.3	1.9	2.2	30
VOL-05D	50.44553	13.20652	718	3.6	4.8	75.0	1.9	1.8	60
VOL-07D	50.44554	13.20653	718	3.5	3.5	100.0	1.4	1.6	40
VOL-38T	50.44128	13.22095	686	4.0	3.5	114.3	2.0	1.0	95

*last letter indicates chromosome number: D – diploid, T – triploid; DBH – diameter at breast height, SQ– slenderness quotient (height to diameter ratio)

crown width were measured by Vertex ultrasonic hypsometer (Haglöf, Sweden) to an accuracy of 0.1 m. Tree foliation (health status indicator) was estimated to the nearest 5% according to methodology used in the international project of ICP-Forests and ICP-Focus (Lorenz 1995). Defoliation was calculated as difference between 100% and foliation. All trees were tested as diploids, while one tree was triploid individual (VOL-38T) (Baláš et al. 2019).

The seeds were stored in cooling box (fridge) at –2 °C. Two variants were tested: non-stratified and stratified. Seeds of stratified variant were first placed in sand and stored in cooling box for one month (cold stratification) before testing. Seeds were not exposed to light prior the experiment. For each tree, 4 transparent plastic boxes (with 100 seeds in one box) with double-layered moisten filter paper (ČSN 50 04) were prepared. For each tree, altogether 400 seeds (200 seeds per variant) were tested, according to ČSN 48 1211 standard. These prepared variants were cultivated in growing chamber (Q-Cell, Poland) with conditions according to standard: temperature 30 °C and light for 8 hours followed by 16 hours in 20 °C and dark.

Number of germinated seeds was counted weekly as number of healthy and fully developed seeds. Germinative energy was evaluated as the counting after first week of experiment. Next three weekly evaluations represented the tests of germinative capacity.

Data analysis. The differences between individual trees and stratification variants in terms of germination were tested in STATISTICA 12 (StatSoft) using nonparametric Mann-Whitney *U* test. Kruskal-Wallis ANOVA test was used to compare the differences in germination of individual trees. Spearman correlation was used to determine the relationship with dendrometric characteristics of trees (height, DBH, SQ, crown base, crown width), health status (foliation) and germination. Statistical differences among data were recorded as follows: significant ($P < 0.05$, $P < 0.01$), marginal ($P < 0.1$) and non-significant ($P > 0.1$). The analysis of the principal components (PCA) was performed in Canoco 5 software (Šmilauer, Lepš 2014) to evaluate the relationship between germination, stratification, tree individuals and tree parameters. Data were log-transformed and standardized before analysis. The results of multidimensional PCA analysis were visualized in the form of ordination diagram.

RESULTS

Germinative capacity and energy

Results of individual trees and variants (stratified × non-stratified) summarized together and compared are in Table 2. In total, 500 seeds (mean 45 ± 37 SD) of non-stratified variant and 518 seeds (mean 47 ± 40 SD) of stratified variant germinated out of initial 2 200 for each variant. Stratified variant showing higher germination in comparison to non-stratified was marked green, and the contrary was marked red in the Table 2. In the first count-

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Table 2. Germination per individual Ojców birch trees differentiated by stratification variants – stratified × non-stratified (stratified variant showing higher germination in comparison to non-stratified is marked green, and the contrary is marked red)

Tree ID	Total initial number per variant	1st week		2nd week		3rd week		4th week	
		non-stratified	stratified	non-stratified	stratified	non-stratified	stratified	non-stratified	stratified
VOL-24D	200	3	0	0	0	0	0	0	0
VOL-16D	200	11	26	1	0	0	0	0	0
VOL-06D	200	71	57	5	5	0	0	0	1
VOL-03D	200	60	58	3	2	0	0	0	0
VOL-15D	200	52	80	4	6	0	0	0	0
VOL-25D	200	58	66	16	2	0	1	0	0
VOL-30D	200	42	53	8	0	0	0	0	0
VOL-32D	200	109	121	10	9	0	0	1	0
VOL-05D	200	18	8	1	1	0	0	1	0
VOL-07D	200	19	21	7	1	0	0	0	0
VOL-38T	200	0	0	0	0	0	0	0	0
Mean		40	45	5	2	0	0	0	0
Median		42	53	4	1	0	0	0	0
SD		30.9	34.6	4.7	2.9	0.0	0.3	0.4	0.3
Total		443	490	55	26	0	1	2	1

SD – standard error

ing, stratified variant performed better in 6 out of 10 cases. In the second counting, it was better only in 3 cases. Statistically, stratification showed no significant ($P > 0.1$) differences between stratification variants in germinative capacity (higher by 0.8% in stratified variant), whereas germination was higher in 1st week (germinative energy) in stratified variants ($P > 0.1$), respectively marginally ($P < 0.1$) higher in 2nd week in non-stratified variant. In stratified variant, 1st week 88.6% of seeds germinated from the total number of germinated seeds, while it was 94.6% in non-stratified variant.

Percentage of germinated healthy seeds for whole period of the experiment per each tree is in Figure 1.

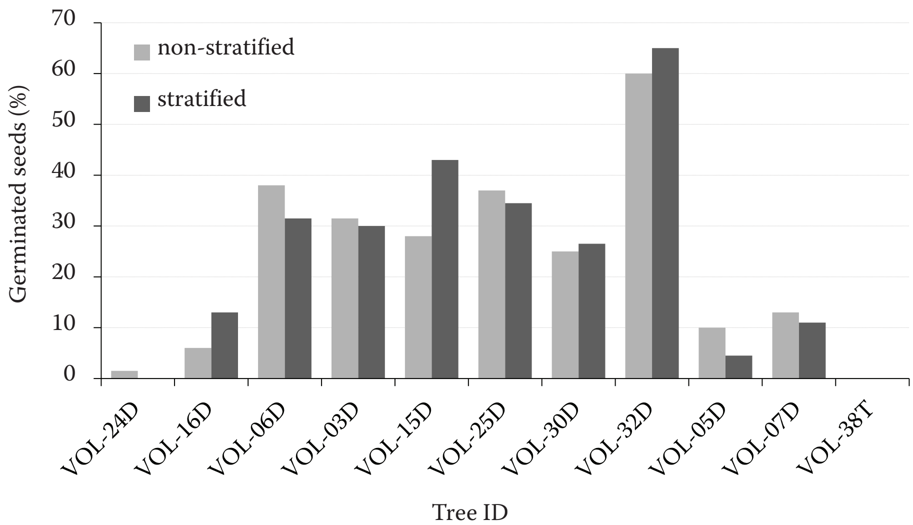

Figure 1. Percentage of germinated seeds for the whole experiment period per individual Ojców birch trees

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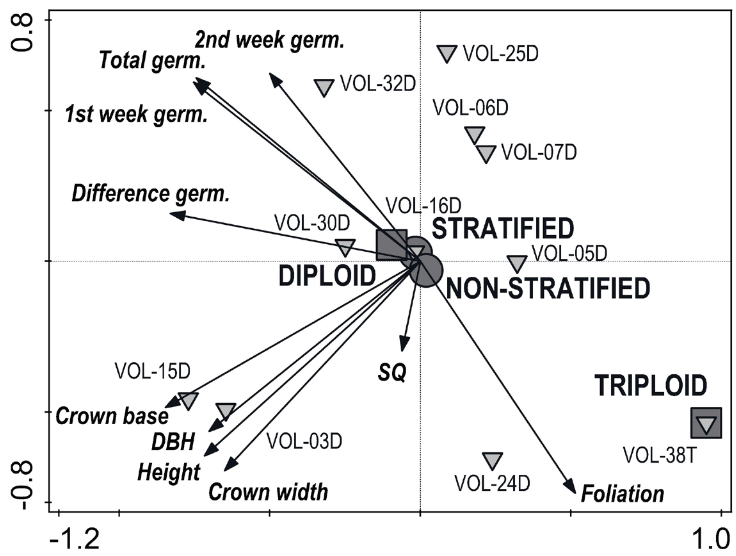

Figure 2. Ordination diagram showing results of principal components analysis of relationship between germination (total, in 1st week, in 2nd week), tree parameters [Height, DBH – diameter at breast height, SQ – slenderness quotient (height to diameter ratio), Crown base, Crown width], health status (Foliation), stratification variants (stratified, non-stratified), set of chromosomes (diploid, triploid) and individual Ojców birch trees

Regardless of variant, most successful in terms of germinative capacity was tree ID VOL-32D (62.5%) followed by VOL-25D (35.8%). Least successful was tree ID VOL-24D (0.8%) because of extremely tiny seeds. Triploid (ID VOL-38T) did not germinate in both variants. Mean germinative capacity for the 11 evaluated tree individuals was 23.1% (22.7% and 23.5% for non-stratified and stratified, respectively). When only diploids were involved, mean germinative capacity was 25.5% (25.0% for non-stratified and 25.9% for stratified seeds). In four tree individuals, stratification improved overall germinative capacity, in 6 trees the number was lower for the stratified variant. Generally, there were significant ($P < 0.01$) differences in germination between individual birch trees from the study site.

Relationship between germination, dendrometric parameters and health status

PCA results expressing the relationship between germinative capacity and energy, tree parameters and foliation of individual trees are presented in the form of ordination diagram in Figure 2. The first ordination axis explains 44.71%, the first two axes 77.40% and the four axes together account for 95.40% of data variability. The x-axis represents germination difference between stratification variants and the y-axis represents the height to diameter ratio. Tree height, DBH and crown parameters were positively correlated to each other. Total germination was positively correlated to germination in 1st and 2nd week, while these parameters were significantly negatively correlated with foliation, respectively positively with defoliation. The lowest explanatory variable in ordination diagram was height to diameter ratio. The differences between tree individuals and set of birch chromosomes (diploid, triploid) were significant, while germination difference between stratified and non-stratified was very low.

Total germination was significantly ($P < 0.01$) correlated especially with germination in 1st week ($r = 0.99$) and in 2nd week ($r = 0.80$; Table 3). Tree parameters (DBH, height, crown width, crown base, HDR) have no significant ($P > 0.1$) effect on germinative capacity and energy, while these parameters (except HDR) were significantly positively ($P < 0.01$) correlated to each other ($r = 0.77–0.93$). On the other site, germination was significantly ($P < 0.01$) negatively correlated with foliation ($r = 0.72$).

DISCUSSION

Generative reproduction is one of possible ways to regenerate rare tree species (Vincent 1965; Grime 2001). However, seed production is dependent on many factors such as mast year, climatic factors and soil conditions (Procházková et al. 2002). In natural condition, success of seed reproduction can be significantly limited due to hares (*Lepus*) and droughts (Rao et al. 2003; Vacek et al. 2018b). On study site Volyně, reproduction is also significantly affected by dense grass cover (Baláš et al. 2016), which does not allow the rooting of the seeds in the soil and subsequently it negatively limits seedlings by strong competition (Vacek et al. 2017c). In controlled conditions, it can be done with success that is however conditioned by germinative energy and germinative capacity. In our

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Table 3. Spearman correlation matrix of seed germination (germ.), Ojców birch tree characteristic and health status; significant differences ($P < 0.01$) are marked by asterisk and green colour

	Total germ.	1st week germ.	2nd week germ.	Height	DBH	HDR	Foliation	Crown base	Crown width
Total germ.	1.00								
1st week germ.	**0.99***	1.00							
2nd week germ.	**0.80***	**0.73***	1.00						
Height	–0.04	0.03	–0.16	1.00					
DBH	0.11	0.17	–0.06	**0.87***	1.00				
HDR	–0.07	–0.06	0.03	0.18	–0.24	1.00			
Foliation	**–0.66***	**–0.66***	**–0.59***	0.09	0.01	0.27	1.00		
Crown base	0.20	0.25	0.07	**0.87***	**0.93***	0.00	–0.05	1.00	
Crown width	0.03	0.06	–0.16	**0.77***	**0.78***	0.02	0.40	**0.72***	1.00

DBH – diameter at breast height, HDR – height to diameter ratio (slenderness quotient)

study, stratified variant of Ojców birch seeds showed higher germinative energy (first counting) and overall higher germinative capacity. However, the differences were relatively small and did not change the germinative dynamics of individual trees. The mean germinative capacity ranged 23.1% which suggests a possibility of generative reproduction in controlled environment.

Seed germinative capacity is highly variable parameter (Hoffman et al. 2005; Debnárová, Šmelková 2008). Juntilla (1970) reported germinative capacity around 75% in dwarf birch (*Betula nana* L.) for seeds exposed to alternating temperatures, but only 10–15% for seeds stratified under a constant temperature. High variability was reported also for paper birch (*Betula papyrifera* Marsh.), as from different provenances it reached 11.1–90.8% (Benowicz et al. 2001). For *Betula pendula*, most similar taxon, the germinative capacity was reported to be between 17.5–48.75% (Reyes et al. 1997), which is comparable to our findings for *Betula oycoviensis*. Tylkowski (2012) emphasized that the germinability of *Betula pendula* is affected by storage time and temperature. For the conditions of our methodology, there should be no negative impacts as seeds were stored around one year in –2 °C. This species supposedly does not require light pre-treatment for full germinability, as some other birches like monarch, Japanese or Erman. (Bonner, Karrfalt 2008). Stratification is recommended, but in our case of *B. oycoviensis*, it did not have a significant effect on germination characteristics.

It is reported from numerous studies that particularly rare plant and tree species have low germinative capacity, especially without suitable stratification treatment (Schubert 1993), as for *Sorbus torminalis* L. (Var et al. 2010) or *Taxus baccata* L. (Melzack et al. 1982). In this context, the results of our study are satisfying, because they suggest the possibility of practical use of reproduction from seeds.

The ability to manage species-rich and stable mixed forests is dependent on many factors like natural regeneration, deer damage (browsing, fraying and bark stripping) and management of disturbances (Simon et al. 2010). In some cases, the ability of natural regeneration of a native and important species that is enriching forests is limited. Ojców birch in Volyně is a typical example. The only confirmed locality in Czech Republic suffers from heavy deer browsing and lack of active silvicultural management. Also, extreme climatic events could possibly be the partial reason of recent decline (Gallo et al. 2014; Mikulenka et al. 2020). Individual Ojców birch trees decline in time and natural regeneration has not occurred (Ondráček 2008). Controlled generative reproduction in combination with artificial regeneration is one of possible ways to conserve the unique birch stands (Košut 1982). Other ways include different types of vegetative reproduction (Vacek et al. 2010; Vítámvás et al. 2020). Our study showed that different seed lots of Ojców birch contain viable seeds that can be used for regeneration and conservation of this species, in our case the germinative capacity was 23%. It is possible that under various other stratification treatments the germination could be boosted, but in our case the stratification did not play a crucial role for the germinative parameters. Vincent (1965) also states regarding birch that stratification is not necessary, but that seed collection must

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be done in the right time and that for good germination the seeds must not dry out.

In terms of tree data, germination of the Ojców birch was negatively correlated with tree foliage i.e. positively with defoliation. Many statistical analyses of time series often show negative correlations between seed production and radial tree growth (Dittmar et al. 2003; Mund et al. 2010), which they refer to as "switching" resource use from vegetative growth for reproduction, especially in strong mast years. Similarly, there was a decrease in foliage in the seed years of European beech compared to the years without fructification (Vacek 1987). This switching of resources does not necessarily reflect resource constraints or compromise between vegetative and reproductive growth or foliage. Rather, it shows that fructification responds to external or internal conditions differently than radial growth or foliation. This may mean that climatic conditions that increase reproductive growth are unfavourable for vegetative growth (radial growth and foliation) (Knops et al. 2007, Hirayama et al. 2008). Also, Innes (1994) and Seidling (2007) report that both the flowering process and the seed formation, especially in strong seed years, reduce leaf biomass. Bonner, Karrfalt (2008) also mentioned that excessive seed production is related to deteriorating crown vitality. On the other hand, no significant correlation of germination of the birch trees with other dendrometric data was found.

CONCLUSION

Ojców birch has an importance in European nature as a rare taxon diversifying European forests and increasing diversity of gene pool in genus *Betula*. At the locations of its occurrence, its vitality and numbers are declining. This study of the germinative capacity and germinative energy tests showed that it can be reproduced generatively with success, which is however dependent on seed vitality of individual trees. Supposedly triploid individual was unambiguously not able to generatively reproduce. Stratification did not show crucial positive effects on the germinative capacity and germinative energy of Ojców birch, even though improving germinative energy. On the other hand, the seed year in connection with germination had a significantly negative effect on birch foliation, as favourable weather conditions in recent years have increased reproductive growth at the expense of vegetative growth. This is a good signal given the possibility of natural regeneration of this rare tree species. Sufficient natural regeneration of this species is a prerequisite for the conservation and successful development of this valuable population.

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