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The Seeds Quality of *Betula pndula* Roth and *Betula oscura* Kotula from Semi-Natural and Anthropogenic Habitats

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Abstract: This research presents the results of the study of seeds quality of two Polish native species of the genus *Betula* L. (*Betula pendula* Roth and *Betula obscura* Kotula), growing on the semi-natural (low human activities) and anthropogenic (high human activities) habitats. In this study the generative phase has been investigated to find differences between studied taxa and try to find out why population of *B. obscura* is very rare. In this research we have checked the quality of the seeds from *B. obscura* and *B. pendula* and test the survival of seedlings sowed into the ground researched in laboratory conditions. Results of germination capacity shown significant difference between trees from the anthropogenic and semi-natural sites; the energy capacity indicated significant difference between trees: *B. pendula* and *B. obscura* from all study areas. Mixed-model ANOVA/MANOVA for seedlings growth showed statistically important effects of factors (species, area, months).The results show that *B. obscura* grows better on the anthropogenic habitats than *B. pendula*, but the latter had more of the fully developed seeds and higher germination and seeds energy capacity.

Key words: Aggregation index, seeds germination capacity, seeds energy capacity, survival of seedlings

INTRODUCTION

One of the most important processes which defines the rapid growth, competition and ability to adapt, is the reproduction factor. The reproduction is decided mostly by fecundity of specimen and also survival rate of the progeny, until it becomes able to reproduce itself (Galloway, 2005; Tang and Tiderstedt, 2001). This variability is conditioned by both genetic and environmental factors. It is so because the number of offspring produced in different conditions is not as important as its ability to survive and reproduce (Sultan, 1995; Callaway *et al*., 2003; Alpert and Simms, 2002).

Betula obscura Kotula is endemic species in Central Europe covering the Czech Republic, Slovakia, Poland and Ukraine (Lesiñski, 1999). Stecki *et al.* (1928) made detailed research morphology and anatomy of these taxa and after checking such qualities as bark, leaves, generative organs and shoots recognized *B. obscura* as a species. Affirmations of this thesis were studies made by Jentys-Szaferowa (1959), where perceived differences between the same traits both for *B. obscura* and for *B. pendula*. *B. obscura* was acknowledged as a species by Szafer *et al.* (1986), Seneta (1973) and Gostyñska-Jakuszewska (1992 cit. Mirek *et al*., 2002). A different point of view had been taken by Seneta and Dolatowski (1991, 2003), Zając and Zając (2003) and Mirek *et al.* (2002) those have acknowledged this taxon as a new form. Rutkowski (2004) recognized *B. obscura* as subspecies.

There is no difference between seeds production by *B. pendula* and *B. obscura* (Jentys-Szaferowa, 1959). However, according to our observations, the nuts sizes of *B. pendula* are bigger. It is assumed that large seed sprout faster than the small ones. The number of sprouts from larger seeds is also bigger, because are better nutritionally supplied (Faliñska, 1996, 1998; Rees and Venable, 2007; Moles and Westoby, 2006; Burley *et al*., 2007). Size and seeds progression has huge influence on their sprouting and further development. The bigger they are the better-developed sprouts will be. Further seedling development depends on the environmental factors, that can by qualified into two classes like natural environment factors (water, temperature, light, moisture, minerals and pH of the soil) and factors caused by human interference-anthropogenic factors (salinity of soil, organic and non-organic chemicals, pesticides, fertilizers) (Franiel and Więski, 2005; Gratani and Bombelli, 2000; Grzesiuk and Kulka, 1981; Loveitt-Doust, 1989; Wei *et al*., 2007; O`Reilly and De Atrip, 2007). These factors decide how the process of ripping and sprouting will develop.

In this research the generative phase of *B. pendula* and *B. obscura* has been researched to find the

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differences between them and try to find out why *B. obscura* as a taxon, has a few representatives. We checked the seeds quality of *B. obscura* and *B. pendula* and tested the mortality of seedlings sowed into the ground in laboratory conditions.

MATERIALS AND METHODS

Study sites: The study population of *Betula pendula* and *Betula obscura* were located in four study plots covering the area of 500 m^2 on the Silesian Upland in southern of Poland, in the moderate climate zone (extreme mean temperatures of 7-8°C and 700-800 mm of average annual rainfall). Two contrasting types of *Betula* L. woodlands can be found in this areas: semi-natural represent by two nature reserves Las Murckowski in Katowice (50°21`N, 19°10`E) and Sasanka in Jaworzno (50°27`N, 19°40`E) and region under anthropogenic pressure represent by the Ponds Paciorkowce in Bieruñ Nowy (50°32`N, 19°24`E) and Mine Sand Szczakowa in Jaworzno (50°32`N, 19°40`E). The soil type in all sites is podsol and rusty with pH 7.0-7.3 (Tokarska-Guzik, 1996; Tokarska-Guzik and Chmura, 1996; Gorczyca *et al*., 1997).

Data sampling: During vegetative seasons 1999-2001, at each study plots 23 mature specimens of *B. pendula* and 15 specimens of *B. obscura* (20-25-years-old) were selected. Ten female fructifications were taken from the mid-crown of each birch tree, situated in a paper bag and transported to the laboratory.

Seeds germination capacity: At the Ecology Department 300 seeds (100 seeds per one repetition) from each investigated tree were placed on tissue paper in 12 Petri dishes. Seeds were kept in growth chamber at a temperature of 24°C for 14 days. They were also irradiated in 24 h cycle: 16/8 h, by light of 880 μEm^{-2} sec^{-1}. The dishes were hydrated with distillated water and the numbers of sprouted seedlings were counted every day. For each study area there were made 3 repetitions, which tested ability of the seeds to sprout after a month.

Seeds energy capacity: The seeds energy was calculated after 14 days. We used formula recommended by Lityñski (1977):

$$W = \Sigma(d \times pd)k^{-1}$$

Where:

W = Pieper factor
d = Next day of sprouting
pd = Percent ratio of seeds sprouted in the following day
k = Percent ratio of all sprouted seeds

Degree of seeds development: One thousand seeds, picked randomly, were taken from each area from *B. pendula* and *B. obscura* pure seeds sample. The observations were made to check degree of seeds development using optical microscope MB 30. Test was supposed to tell, whether seeds were full, partly-fill embryo, full seed not fertilized or empty (Noland *et al*., 2006).

Seedlings survival: The surface layer of soil from all study areas was carried into Ecology Department Growth Room and put into 80 (2 species × 4 study area soil × 10 plastic pots: 8×7×7 cm) pots. Seeds from both species germinated in growth chamber and after 14 days, 800 seedlings were planted out to the pots. Seedlings were hydrated distillated water and every week their number was checked. After 150 days the experiment was terminated.

Statistical analyses: Simple statistical parameters (mean, standard deviation) for all vegetative period were estimated by using the Descriptive Statistics procedure of the Statistica 5.1 PL, StatSoft, Poland. The variation of germination and seeds energy capacity between the study areas was evaluated by the Kruskal-Wallis test with the non-parametric procedure, because result of Shapiro-Wilk test did not show a normal layout. Mixed-model ANOVA/MANOVA was using to detected significant differences and effect interaction in percentage of seedling survival between study area taxons and months. Seedlings survival comparison between two taxons and two types of habitats were determined by chi square test (Zar, 1999; Stanisz-Wallis, 2005).

The aggregate measurement was used to define, which study areas displays the best habitat conditions to develop of birch seedling. It was done by calculating average arithmetic from each variable(stimulants: seed germination capacity, energy, percent of fulfilled seeds, percent of surviving seedlings and distimulants: percent of empty seeds, percent of seedlings not survive), which were made comparable thanks to unifying them and expressing this average on point scale in the range from 0 to 100 (Thiele, 1993). The formula then takes the form:

$$W_i = 100 \, (m \bullet \Sigma \, \alpha_j \, x'_{ij})^{-1}$$

Where:

W_i = Aggregation index

m = No. of traits
α_i = Weight of j-variable
x'_{ij} = Stimulants or distimulants

The sum was made by unifying with the use of these formulas:

Stimulants: $x' = (x_{ij}-\min\{x_{ij}\})/(\max\{x_{ij}\}-\min\{x_{ij}\})$
Distimulants: $x' = (\max\{x_{ij}\}-x_{ij})/(\max\{x_{ij}\}-\min\{x_{ij}\})$

RESULTS AND DISCUSSION

Analysis of the results of germination capacity shown significant difference between trees from the anthropogenic and semi-natural sites (df = 8; H = 18.67; p = 0.016). Results of the energy capacity indicated significant difference between trees: *B. pendula* and *B. obscura* from all study areas (df = 8; H = 16.08; p = 0.04) (Fig. 1). Seeds of *B. pendula* characterized a high degree of development, because more than half of them were fully developed and this vegetative season we can define as a highly productive (Fig. 2). Mixed-model ANOVA/MANOVA for seedlings growth showed statistically important effects of factors (species, area, months) (Table 1).

The aggregate index showed that the best conditions for *B. pendula* can be found on the Ponds Paciorkowce, where Wi index amount 35.7, while the worst was on the Mine Sand Szczakowa (Wi = 16.5) and creates the best conditions for *B. obscura* (Wi = 41.9) (Fig. 3). In both reserves the habitat conditions was very similar. The habitat conditions were not good enough for the seeds quality of *B. obscura* in Las Murckowski (Wi = 27.7) and Sasanka (Wi = 22.6).

After taking into consideration two populations: *B. pendula* and *B. obscura*, generally the percentage of seedlings survival in the end of the study (150 days) was 62.5% for *B. pendula*, while for population of *B. obscura* 88.5%. The results Chi-square test separately for semi-natural and anthropogenic areas, the acquired result was very highly statistically important, if considered as the data about survival of seedlings (Chi-square = 8.42, df =1, p = 0.0037).

The evaluation of seeds vitally and condition are very important research and practical problem. In case of the investigation of seed germination capacity, germination tests used these days are accurate, lengthy procedures. A statistically significant in the dynamic of sprouting seeds each site was received. It would seem that the biggest amount of sprouted seeds was on the semi-natural habitats (reserve Las Murckowski and reserve Sasanka). This sites have nearly background levels of pollution and studied taxons in these sites look healthy. The anthropogenic habitats represent heavily polluted industrial barrens, where *B. pendula* and also

Table 1: The seedlings survival rate of *Betula pendula* and *Betula obscura* in pot experiment, using mixed-model ANOVA/MANOVA (N = 800, p<0.01)

Effect combination
1-species; 2-area; 3-month

Effect	df effect	MS effect	df effect error	MS effect error	F	p
1	1	0.860	152	0.064	13.309	<0.01
2	2	0.977	152	0.064	15.131	<0.01
3	3	0.535	152	0.064	8.282	<0.01
1,2	3	2.492	152	0.064	38.579	<0.01
1,3	2	0.269	152	0.064	4.176	<0.01
2,3	6	0.767	152	0.064	11.874	<0.01
1,2,3	6	0.930	152	0.064	14.404	<0.01

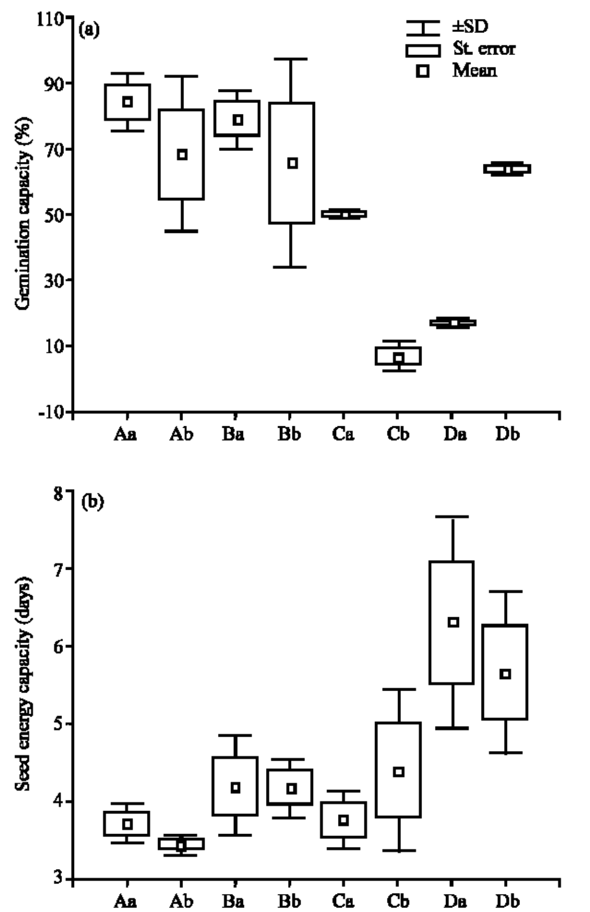

Fig. 1: Results of Kruskal-Wallis test for testing effect (a) germination capacity and (b) energy capacity between semi-natural habitats (A - reserve Las Murckowski, B-reserve Sasanka), anthropogenic habitats (C- ponds Paciorkowce, D-mine sand Szczakowa) and species (a-*Betula pendula*, b-*Betula obscura*)

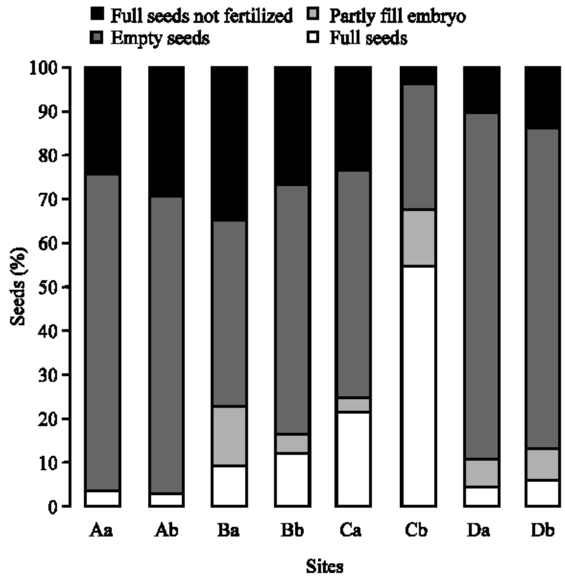

Fig. 2: Percentage of seeds development of *Betula pendula* (a) and *Betula obscura* (b) in all study sites: A-Reserve Las Murckowski, B-Reserve Sasanka, C-Ponds Paciorkowce, D-Mine Sand Szczakowa

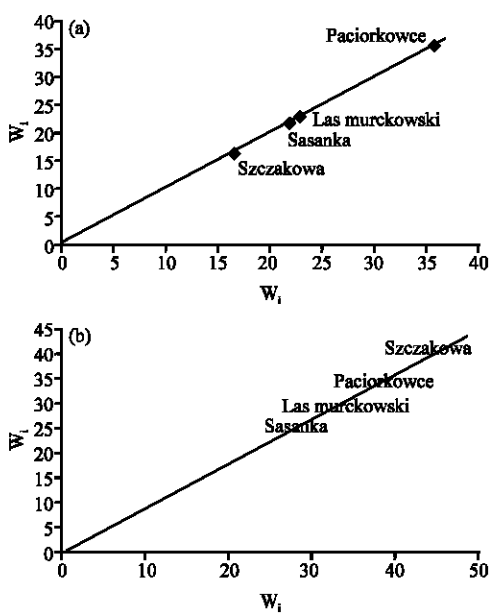

Fig. 3: The aggregate index (Wi) of studied areas for (a) *B. pendula* and (b) *B. obscura*

B. obscura are quite infrequent. The survivors demonstrate by a devoid of leaves, discoloration and a low number of fruits. High level of pollution emitted by local industry (coal-mine and power plant) has caused widespread destruction of soils and vegetation (Tokarska-Guzik, 1996; Tokarska-Guzik and Chmura, 1996; Gorczyca *et al*., 1997; Kozlov and Niemelä, 1999; Valkama and Kozlov, 2001; Zvereva and Kozlov, 2005). This is a main reason decrease of seeds quality. Seeds collected from Szczakowa and Paciorkowce characterized by a low germination capacity, which probably is an effect of the specimen's bad condition, cause by a vicinity of plants and their intensive activity. It can be assumed that habitat conditions have huge influence on the dynamic of seeds sprouting both for *B. pendula* and *B. obscura*. However, without distinction of the study area *B. pendula* showed better ability to sprout than the *B. obscura*.

One of the parameters characterizing vitality and seeds condition is their germination energy. High speed of sprouting is a good indicator of seeds vitality, but does not show the seeds ability to sprout into the matured plant (Osumi and Sakurai, 1997). The results of sprouted seeds give only quick information about the sowing material. It becomes important to obtain the knowledge about sprouting energy of the seeds from each probe, counted as a Pieper factor. The higher factor is the lower speed of sprouting (Lityński, 1977). The biggest Pieper factor had the birches growing in Szczakowa, which explains their low dynamic of sprouting. The sprouting energy of other birches was more or less on the same level.

The seeds quality depends on the habitat conditions and maturing of the mother plant. On the process of seeds forming, large effect has environmental conditions (Donohne, 2003; Galloway, 2005; Marchoil *et al*., 1999). The point of the process was to qualify seeds as full, half-full, empty and partially developed. Seed was acknowledged as full only if they had fully developed germ, which occupied the whole space of the seed and only those seeds were able to sprout (Noland *et al*., 2006). There was a large disproportion between full and empty seeds, both *B. pendula* and *B. obscura*. However, seeds of *B. pendula* were better develop and concurrently had better germination and energy capacity. The reasons for badly developed germ can be a low temperature during the period of the blossom, pests or sickness of the plant (Grzesiuk, 1965; Osumi and Sakurai, 1997).

The habitat conditions are important for dynamic of seeds sprouting and on the development of the seedlings. *B. pendula* is characterized by a wide ecological amplitude and high tolerance on the habitat conditions (Franiel and Wieski, 2005; Franiel, 1996). It is the reason of its wide range appearance. Observation of *B. obscura*, suggest that in her neighbourhood can be found *B. pendula* which usually exceeds *B. obscura* in the number of specimen (Sokołowski, 1966; Fiołkowa, 1974; Danielewicz, 1990; Łaszek, 1993). It is then possible that the both trees compete against each other. Competition narrows down the boarders of the plant tolerance (Chengs *et al*., 2005; Kreyer and Zerbe, 2006; Lehvaervirta and Rita, 2002). After getting rid of the concurrent, some species can show wider ecological amplitude then in the presence of the other plants (Falińska, 1998; Tobe and Gao, 2007). It is possible that after getting rid of *B. pendula*, the range of distribution of *B. obscura* would be wider. Most probably the selection mechanism acting in anthropogenic habitat allows survival of individuals of both birches different reproductive strategies. Seeds starting to sprout in the first and second season, while the contribution of the last one is smaller, which suggests the dynamics changes in the selection pressure manifested stronger in the environment where changes in biotic and abiotic factors are not gradual but jump wise. It can be noted even within the same vegetation season. Such populations cannot be unambiguously placed in the continuum of life strategies. The set of these features suggests that the selection pressures at the moment of transformation of pioneer habitats into stable ones do not change gradually but jump wise. I certain environments these changes can be characterized by strong dynamics detectable even within the same vegetation season (Alpert and Simms, 2002; Falińska, 1998).

After taking into consideration all studied germination features, it would seem that *B. obscura* shows lower level of variability on the anthropogenic areas than the *B. pendula*. It seems that *B. obscura* is as resilient to pollution as *B. pendula*. By using the aggregate index studied areas were divided by the means of the best environmental conditions for living on them species of birches. For *B. obscura* the best condition are on the areas under anthropogenic influence, where as for *B. pendula* these conditions are not very important for its development. This study proves that *B. obscura* is more resilient to hard environmental conditions, in comparison to the *B. pendula*. It is possible that if there would not be competition between those species, the amount of specimen of the *B. obscura* would be as high as silver birch trees.

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