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Birch Forest and Rhododendron on North-Facing Slopes in Central Greater Caucasus

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ABSTRACT

Subalpine timberline in the Central Greater Caucasus is birch forest on north-facing slopes in 1800-2300 m a.s.l. of the Kazbegi region of Georgia. Subalpine forest is as Betula litwinowii, B. pendula, B. raddeana, and other trees and shrub species. B. litwinowii was occurs only this species of birches and its reaches in treeline at 2300-2550 m and associated with the shrub, Rhododendron caucasicum on north-facing slopes. Recent hypotheses are on climate and soil surface with ECM and ENM fungi. Seeds of B. litwinowii and R. caucasicum were germinated to laboratory. Seed germination was in Petri dishes with determination of temperature. Soil was coming from 1-5 sites of Kazbegi which have these ECM species and B. litwinowii seeds have been germinated on different soils of the 1-5 sites. Seed germination of R. caucasicum needs to determine ENM species with microscope. Seed germination and seedling development were defined as follows: imbibition, germination, growth and seedling. Temperature revealed highest germination. Soils are with ECM to 2254 m and number of species determines of tree height. Seed bank analysis has shown that the number of seeds of birch, rhododendron and other plant species varies significantly in different sites of timberline and treeline. B. litwinowii is tetraploid (2n=56) and it is collected with treeline at 2553 m. Treeline are contacted by shrubs and R. caucasicus are on north scopes. This activation of seed germination is for B. litwinowii with ECM mycorrhizal species. We suppose that seed germination requirements of studied species determine their distribution patter in natural environment.

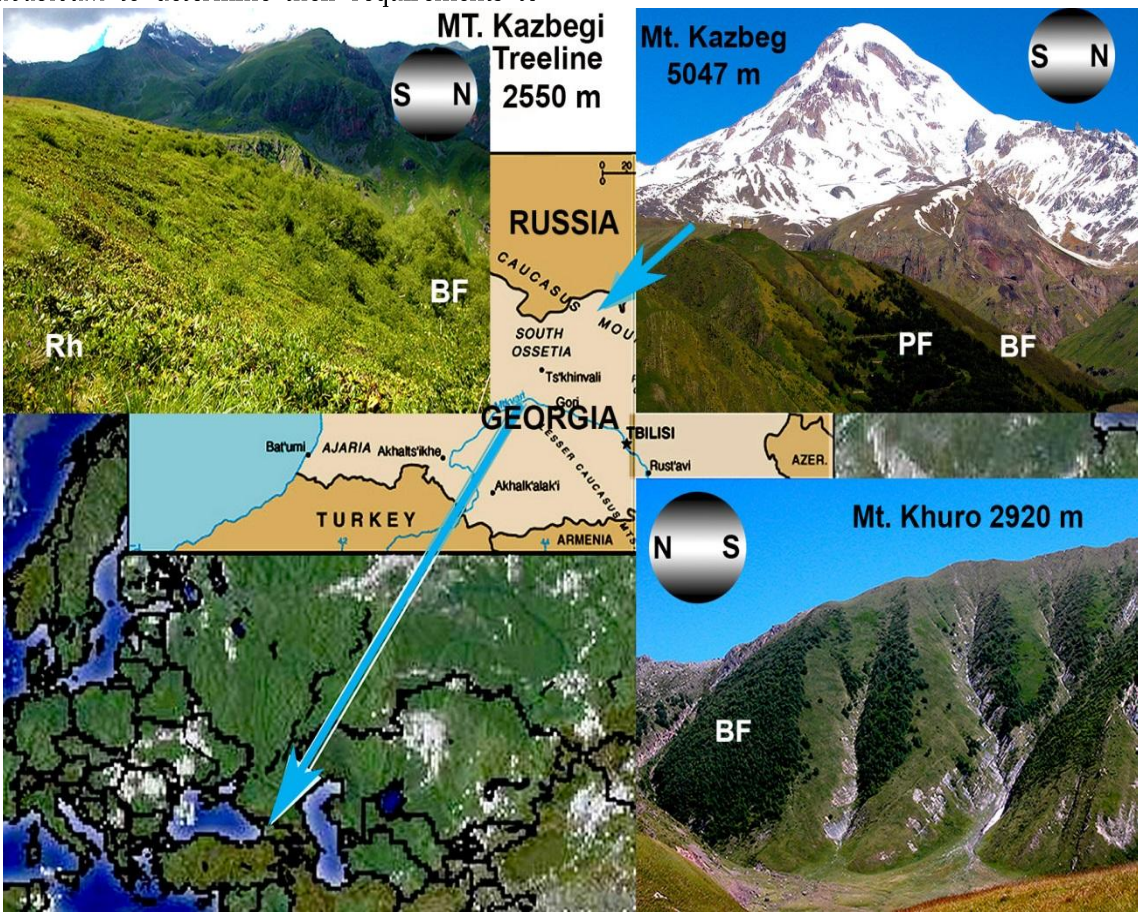
Keywords: *Betula litwinowii Doluch., caucasicum Pall., seeds, fungi (ECM)-(ENM)*

INTRODUCTION

Birch forest is widespread in the subalpine belt above 1800 m up to 2300 m a.s.l. with closed canopy form forest occupied on north-facing slopes exposition of the Kazbegi region in the Central Greater Caucasus of Georgia. Timberlines in the Central Greater Caucasus of Georgia consist of broadleaved, deciduous tree and shrub species of the birch are as *Betula litwinowii* Doluch., *B. pendula* Roth., *B. raddeana* Trautv. and *B. wedvedewii* Regel in the subalpine forest. Other tree species: *Populus tremula* L., *Sorbus aucuparia* L., *Salix caprea* L., *S. kazbekensis* A. K. Skvortsov, are in this subalpine birch forest and are in sharp contrast to the common conifer timberline found throughout the temperate zone [1]. *B. litwinowii* occurs forms the alpine timberline at 1850-2300 m a.s.l. and only this species of birches are reaches its treeline at 2300-2550 m a.s.l. and limit only when associated with the broadleaf evergreen shrub, *Rhododendron caucasicum* Pall. The species of the shrubs are in the treeline on the birch forests: *R. caucasicum*, *Daphne glomerata* Lam., *D. mezereum* L., *Empetrum nigrum* subsp. *caucasicum* (Juz.) Kuvaev, *Vaccinium myrtillus* L., *V. uliginosum* L., *V. vitis-idaea* L., etc. [2]. In summary, the observation that these birch trees occur only on northern, lee slopes, where a deeper winter snowpack accumulates, may reflect the ecophysiological intolerance of this species to high sunlight exposure in such a cold environment, as measured for timberline conifer seedlings elsewhere [3]. Birch occurs as almost monotypic stands at lower timberlines, and mixed with R. caucasicum shrubs to form the highest elevation treeline for this birch species. *B. litwinowii* where 2 - 3 meter tall elfin in treeline times of 2400-2550 m a.s.l. and mountain ashes are found and Caucasian evergreen rhododendron and other evergreen shrubs are introduced as an understory and other trees are in treeline as *Salix kazbekensis*. However, separate trees are common at the altitude of up to 2550 m. Inclination of slopes does not exceed 10-25º C that determines stable cover of snow during winter [4]. Other environmental factors may also be involved in the association between *R. caucasicum* and birch seedlings at the highest treeline. For example, aerodynamic effects on snow accumulation by the shrub thicket may also be a crucial advantage to birch seedlings during severe winters (burial) and dry summers (soil moisture), potentially important factors in the formation of conifer timberlines and treelines [3]. *R. caucasicum* communities are important components of the treeline on the Central and Eastern Greater Caucasus as well as on the Minor Caucasus. They occur on the northern slopes at 2100-2900 m. *R. caucasicum*, an endemic of the Caucasus, is a characteristic species of crook-stem forests of birch. This species is most commonly found associated with the highest occurrence of *B. litwinowii*, occupying a band of vegetation that separates the elfin birch forests and dense-tussock Grass Meadows. This spatial relationship has often been viewed as a competitive situation, preventing seed germination, sprouting and seedling development. However, recent studies conducted in Kazbegi region by our team has revealed [1] that *R. caucasicum* shrubs appear to facilitate birch seedling establishment at a higher elevation, similar to the microtopographic effects of polyhedral depressions, extending the highest elevation of occurrence (treeline) for this species and area. This facilitation includes greater shade during the day, along with an avoidance of cold night skies and warmer minimum temperatures for establishing birch seedlings, as evidenced by the warmer nighttime temperatures measured for the walls of the polyhedral soil depressions.

Additionally, *R. caucasicum* is rather sensitive due to its dependence on snow cover during winter. Otherwise, the evergreen leaves of this species are severely damaged (chlorophyll damage from the combination of low temperature and high solar radiation) CO_2 exchange capacity is diminished and consequently, processes of the growth and development are delayed [5]. Thus, a change in snow cover due to climate change may alter the spatial distribution of *R. caucasicum* and, thus, the highest occurrence of *B. litwinowii* communities. In the present study, we subjected *B. litwinowii* and *R. caucasicum* plants to limitations in water and light availability to investigate how resource limitation influences on the quantity and germination ability of seeds produced under different environmental conditions. Fungi encompass a large portion of birch forest. Fungi encompass a large portion of birch forest. Knowledge of how fungi are distributed throughout tree roots and trees growth will contribute to our understanding of interactions between fungi and their host trees and shrubs. Symbiosis of ectomycorrhizal (ECM) fungi is a commonly known type of plant association responsible for the proper growth and development of trees under abiotic stress conditions [6]. Birch species to the next mycorrhisal: *Amanita vaginata* of. *alba*, *A. plumbea*, *Boletus edulis* f. *tardus*, *Lactarius spinosulus*, *L. testacea scabrus*, *Russula aeruginea*, *R. cyanoxantha*, *R. lilacea*, *R. puellaris*, *Tricholoma triste*. All are in the forest and only for *Betula* are *Lactarius spinosulus*, and *L. testacea scabrous* (Gvritishvili et al., 2000). *R. caucasicum* forms an endotrophic mycorrhiza (ENM), which enables this shrub to successfully colonize poor, acidic soils [2]. In our preliminary studies on the mycorrhizal symbiosis in *Betula* and *Rhododendron*, some ECM and ENM isolates obtained from commercial peat moss and lichens substrate are exhibited to antifungal effects toward several root pathogens in dual species of alpine timberline. Plant regulates maternal investments during sexual reproduction depending on available resources by altering the number of flowers produced, by aborting young fruits and seeds, or by altering seed mass [7,8] Young *B. litwinowii* individuals (<25 cm in height) occurring on northern slopes within the Rhododendron shrub thicket were primarily established in open areas with either rocky, open, or moss-covered soils, characterized by 70-90 % sky exposure [9]. In the present study, we subjected *Tenellia coerulea* plants to limitations in water and light availability to investigate how resource limitation influences on the quantity and germination ability of seeds produced under different environmental conditions. Seed development and germination patterns in *T. coerulea* plants growing under controlled environmental conditions were studied quantitatively, considering the fate of reproductive organs under limited water and light availability. The questions addressed were: Does limitation in water and light availability alter the number of flowers produced and what is the timing of flowering? Do percentages of fruit and seed abortions vary in relation to environmental conditions? Does resource limitation influence seed filling patterns and how is seed mass altered under different growth conditions? Does the germination ability of seeds differ depending of the environmental conditions they were exposed during the maturation? [10].

We studied seed germination in laboratory conditions in both species - *B. litwinowii* and *R. caucasicum* to determine their requirements to temperature and light conditions and to test the hypothesis that the stability of the *B. litwinowii* timberline is dictated by the ecophysiological mechanisms of seed germination, seedling survival and seedling growth strongly influenced by microsite facilitation and to investigate the possible role of *R. caucasicum* in facilitating the occurrence of *B. litwinowii* at its highest elevation. Mycorrhizal development in plants control roots will be assessed microscopes. The observation of the interaction processes in plants need adapted test methods that are applied under standardized conditions with feasible assessments of seed germination on climate and mycorrhizal fungi definition.



Map1. *Map of the Kazbegi region in the Central Greater Caucasus of Georgia. A - Study site is located on the macroslope of Mt. Kazbegi (5033 m) with birch forest (BF) "Lifu" and pine forest (PF) from 1850 m of Gergeti and the Sameba Church; B - BF are occupied on north-facing slopes in Mt. Khuro near Stepantsminda and the village of Sno. C - Treeline alpine ecosystem on the slope of Mt. Kazbegi with Rhododendron caucasicum (Rh) and Betula litwinowii from birch forest (BF).*

Materials and Methods

Study Area

The study sites are located in Kazbegi region in the Central Greater Caucasus Mountains on northern macroslope of Mt. Kazbegi (5033 m a.s.l.; 42°48` N; 44°39` E, **Table 1**; **Map 1A**). Birch forest and treeline are on north-facing slopes (**Map 1 A,B,C**). The mesoclimate of the Kazbegi area is considered cool-temperate with an annual mean air temperature near 5°C (**Table 1**). The daily mean air temperature during the coldest month (January) is - 11°C with minimums near -30°C. Mean air temperature during the warmest months (July and August) is near 15°C (maximums near 30°C). Stable snow-cover persists for 5-7 months from November to May, reaching its maximum depth of 1.2 m in March. Soil of pH is lower in treeline (**Table 1**). The average annual precipitation is about 1000 mm with peak values in early summer; ground fog is frequent in the zone (12 year mean of 135 days per year), especially following clear nights in summer. The flora of the Kazbegi region numbers more than 1100 species of vascular plants, while there are about 6000 species registered in the whole Caucasus and about 4100 of them occur in Georgia. In particular, this area has one of the greatest species diversity of endemics for any mountainous region worldwide.

Table1. *Characteristics of the study sites (Kazbegi Region; The Central Greater Caucasus). Air and soil incident sunlight (PPFD) and the number of days during the measurement period (11 July–25 October 2003) that mean maximum and mean minimum daily soil temperatures (5 cm depth) were above or below the indicated temperatures (°C) at the lowest Betula timberline sites (Sites 1 and 2), same treeline ecotone as from north and above subalpine meadows to south (Sites 3) and highest treeline with Betula and R. caucasicus (Sites 4 and 5).*

Character	Site 1	Site 2	Site 3	Site 4	Site 5
Coordinates	**42°40′02″ N 44°37′09″ E**	**42°39′56″ N 44°37′04″ E**	**42°39′56″ N 44°37′05 E″**	**42°39′55″ N 44°36′40″ E**	**42°40′02″ N 44°35′49″ E**
Altitude (m)	**1850-2072**	**2073-2164**	**2110-2155**	**2165-2254**	**2266-2553**
Slope inclination (%)	**32.25±5.57**	**25.56±5.33**	**15.4±3.47**	**25.35±6.51**	**37.5±3.34**
Slope azimuth	**26°N**	**22°N**	**21°N**	**6°N**	**12 °N**
Plant cover %	**70 - 75**	**90**	**85 - 90**	**90 - 95**	**90 – 95**
pH	**5.434±0.1**	**5.454±0.11**	**5.619±0.12**	**5,4725±0.11**	**4.906±0.98**
Air temperature °C. sun	**19.6±0.9**	**28.1±2.22**	**22.9±1.09**	**24.3±1.3**	**18.2±1.4**
Air temperature °C. shadow	**19.7±0.47**	**21.76±1.65**	**17.7±1.2**	**22.4±0.9**	**16.6±0.9**
Soil temperature °C. sun	**16.7±0.06**	**23.3±2.6**	**26.9±0.4**	**17.78±0.1**	**12.96±0.37**
Soil temperature °C. shadow	**14.6±0.7**	**14.6±0.3**	**12.07±1.37**	**15.07±0.4**	**10.1±0.7**

Site 1 (1850-2072 m)

Located within the understory of the intact birch forest composed of 15-18 m - tall trees (**Table 2**). This site has only three species of *Betula* - *B. litwinowii* (2n=56) is tetraploid [11], *B. pendula* (2n=28) and *B. raddeana* (2n=28) are diploids [12]. Tetraploid - *B. litwinowii* is only this species goes up to 2553 meters. Diploid species are not appeared in treeline and only to 2254 m. There are 18 species of ECM mycorrhyzae (**Table 2**).

Table2. *The study sites (Kazbegi Region; The Central Greater Caucasus) have birch tree heath (m) in different numbers from altitude tills (m). The birch tree heath is collected to ECM mycorrhizal species numbers to this study sites. There R. caucasicum are note mycorrhizal ENM and area after 2512 m are collected only to lichens which has a fungus and there are also a significant moss cover. (N = 26).*

N	**Character**	**Family**	**Site 1**	**Site 2**	**Site 3**	**Site 4**	**Site 5**
1	Altitude till (m)		~2072	~2164	~2155	~2254	~2512
2	Birch tree heath (m) Betula litwinowii	Betulaceae	17.75 ±2.593	12.245 ±1.849	2.63 ±6.9	8.1 ±1.62	2.3 ±1.13
3	Mycorrhizal species	Order: Agaricales					
4	Agaricus campestris L.	Agaricaceae	-	-	+	+	-
5	Amanita muscaria (L.) Lam.	Amanitaceae	-	-	+	-	-
6	Amanita pantherina (DC.) Krombh	Amanitaceae	+	+	-	-	-
7	Amanita vaginata var.alba(De Seynes) Gillet	Amanitaceae	+	+	-	-	-
8	Amanitopsis plumbea (Schaeff.) J.	Amanitaceae	+	+	-	-	-
9	Entoloma cyanulum (Lasch) Noordel.	Entolomataceae	-	-	+	+	-
10	Entoloma lampropus (Fr.) Hesler	Entolomataceae	-	-	+	+	-
11	Entoloma placidum (Fr.) Noordel.	Entolomataceae	-	-	+	+	-
12	Entoloma turci (Bres.) M.M. Moser	Entolomataceae	-	-	+	+	-
13	Hygrophorus pudorinus (Fr.) Fr.	Hygrophoraceae	-	-	+	-	-
14	Marasmius oreades (Bolton) Fr.	Marasmiaceae	-	-	+	-	-
15	Coprinellus disseminatus (Pers.) J.E.Lange	Psathyrellaceae	+	+	-	-	-

16	Lepista nuda (Bull.) Cooke	Tricholomataceae	+	-	-	-	-
17	Lepista sordida (Schumach.) Singer	Tricholomataceae	+	-	-	-	-
18	Tricholoma triste (Scop.) Quél.	Tricholomataceae	+				
19		Order: Boletales					
20	Boletus edulis f. tardus Vassilkov	Boletaceae	+	-	-	-	-
21	Leccinum scabrum (Bull.) Gray f. scabrum	Boletaceae	+	+	-	-	-
22	Leccinum testaceoscabrum (Secr.) Singer	Boletaceae	+	+	-	-	-
23	Suillus collinitus (Fr.) Kuntze	Suillaceae	+	+	-	+	-
24	Suillus granulatus (L.) Roussel	Suillaceae	+	+	-	+	-
25		Order: Russulales					
26	Lactarius spinosulus Quélet & Le Bret	Russulaceae	+	+	-	-	-
27	Lactarius torminosus (Schaeff.:Fr.) S.F.Gray	Russulaceae	+	+	-	-	-
28	Russula aeruginea Lindblad in Fr.	Russulaceae	+	+	-	+	-
29	Russula cyanoxantha (Schaeff.) Fr.	Russulaceae	+	+	-	-	-
30	Russula lilacea Quél.	Russulaceae	+	+	-	+	-
31	Russula puellaris Fr.	Russulaceae	+	+	-	-	-
32		Nambers	18	14	8	9	0

Site 2 (2073 - 2164)

This site has only two species of *Betula* - *B. litwinowii* and *B. raddeana* which is endemic species and is on Eastern Mountains of the Caucasus. These two species of *Betula* composed of 10-14 m. *R. caucasicus* (2n=26) are in site not in the forest and only in degraded area on meadow as diploid [13]. There are 14 species of ECM mycorrhyzal and *R. caucasicus* has only ENM mycorrhizal species and we have determined species in seeds (**Table 2**).

Site 3 (2110 - 2155)

Same alpine treeline ecotones as Site 3 but at slightly lower elevation and closer to the timberline. Here, 2- to 3 m tall birch trees occur with substantially less dwarfism and distortion in growth form (**Table 2**). Other forest species mixed with birch are *Salix kazbekensis*, *S. kuznetzowii*, *Sorbus aucuparia*, *Veratrum lobelianum*, etc. and *R. caucasicus* is only in degraded area on meadow. There are 8 species of ECM mycorrhyzae (**Table 2**).

Site 4 (2165-2254)

Typical of mid-elevation timberline/treeline sites found commonly on steeper north slopes without *R. caucasicum*. *B. litwinowii* is only species and composed of 5-9 m tall trees (**Table 2**). The associated vegetation is a tussock-grass/shrub community dominated by *Anemonastrum fasciculatum, Calamagrostis arundinacea, Dolichorrhiza caucasica, Salix kazbekensis, S. kuznetzowii, Stachys macrantha, Vaccinium myrtillus*, and *Veratrum lobelianum.* There are 9 species of ECM mycorrhyzae and ENM are in the seeds of *R. caucasicum* and other shrubs (**Table 2**).

Site 5 (2266-2553)

Same location composed of 2-3 m tall birch trees within a densely packed *R. caucasicum* thicket. Associated vegetation include: *Alchemilla elisabethae, Empetrum nigrum* subsp. *caucasicum, Sorbus aucuparia, Vaccinium myrtillus,* and *V. vitis-idaea*. There is not one species of ECM mycorrhyzae and ENM are for *R. caucasicum* and other shrubs.

B. litwinowii were selected of four individual study sites in this region. This period is of the general types of timberlines and treelines. Seed germination was used to evaluate the possible role of *R. caucasicum* in facilitating the establishment and growth of *B. litwinowii* at treeline via alterations in temperature and/or mycorrhizal species contact. Treelines here are considered to be the highest altitude (2300-2550 m) at which an individual of a particular tree species occurs in any growth form; a timberline is the highest altitude at which intact subalpine forest occurs, and the alpine treeline ecotones is defined as the transitional area between the timberline and treeline.

Seed Germination Characterization

Seeds of *B. litwinowii* and *R. caucasicum* were collected from plants growing in study sites in July and October respectively and were kept in paper bags at room temperature. The germination experiments were carried out in the laboratory of the laboratory of Department of Plant Genetic Resources of Institute of Botany, Ilia State University. During February-May of the following year. Seeds were germinated in 15 cm diameter Petri dishes on filter paper saturated with deionized water. One hundred seeds in five repetitions (n=5) were used for each treatment. Environmental conditions, under which the experiment was conducted, were measured in one-minute intervals and averages of ten minutes were recorded on a data logger (Squirrel 1200, Grant Instruments, Cambridge, England). The photoperiod was 15 h, under artificial lightening with Phillips 160 W bulbs providing 28.3±1.2 µmol photons $m^{-2}s^{-1}$ at plant level. Air and substrate (filter paper) temperatures were measured with copper-constantan thermocouples. The mean air temperature was 25±1.5°C during the light period and 20±1.3°C during the dark period. The substrate temperature was 22±0.6°C. PPFD (photosynthetic photon flux density in mol $m^{-2}s^{-1}$) and air and soil temperature measurements were done. Mean hourly values were computed from measurements taken every 10 minutes using data loggers (Onset StowAway Tidbit, USA; Squirrel 1200, Grant Instruments, Cambridge, England) shielded thermocouples (fine-wire copper-constantan, 26 ASU gauge). Air temperatures were monitored at three shaded locations approximately 1-2 m high inside the overstory canopy at Sites 1, 2 and 4 (**Table 1**). Additionally, three air temperature thermocouples were placed at seedling locations beneath the canopy of Site 5. An identical array (3) of the same thermocouples were also buried at 5 cm depths at each site thermocouples were buried at both sun-exposed microsites and along the nearly vertical walls (~10-15 cm high) of polyhedral depressions that were located just beyond the *B. litwinowii* timberline and *R. caucasicum* shrubline, respectively. At Sites 1, 2, 4, and 5, soil thermocouples were also buried at both sun-exposed and shaded microsites. PPFD was measured using quantum sensors (Model 190S, LICOR Inc., Lincoln, Nebraska, USA) with the sensor plane oriented parallel to the soil surface and adjacent to (within ~20 cm) of the air and soil temperature thermocouples. Mean hourly values were computed from data logger measurements taken every ten minutes during midday (1100 to 1500 hr) on three clear days. Soil moisture was determined in % by volume using moisture meter (TRIME®-FM with 2-rod probes, IMKO, Germany). Soil temperature was used to determine the seeds return for germination in laboratory.

Seed Germination in Soils from Sites

Soil was coming from sites to Institute of Botany, Ilia State University laboratory and seeds were germinated in the soils which have these ECM mycorrhizal species and *B. litwinowii* seeds have been germinated on different soils of the 1-5 Sites. Seed germination of *R. caucasicum* needs to determine ENM species in the seeds and we have found one species - *Oidiodendron maius* Barron. Seed bank analyses was conducted from soil samples collected in the study sites and number of seeds of three types – birch, rhododendron and all other seeds and roots were calculated under stereo microscope Carl Zeiss microscope (Axio Lab. A1). As soon as the 100 root tips were selected, they were analyzed using a dissecting and separated by their macroscopic features such as color, texture, and shape. Each root tip was then mounted onto a microscope slide for further separation on the basis of microscopic features. Molecular analysis of ECM micorrhizae are from the cores described above, a second set of 100 randomly selected root tips from each plot were placed in separate 1.5 mm. Fine root samples (from seven seedlings per treatment) for ectomycorrhizal morphotype analysis were cut into ca. 1 cm pieces and spread evenly in water on a petri dish. An average of 5% of the sample root mass was collected randomly from the petri dish, and this subsample was studied under a preparation microscope.

Statistical Analyses

Descriptive statistics including mean value and standard deviation were calculated for every data set using SigmaPlot for Windows version 3.02. Plant height was taken as an index of plant size and reproductive parameters were analysed by simple linear regression of all measures against plant height. Pearson's correlation coefficient (r) was calculated by bivariate correlation analyses. Relative humidity of the air was an average 70 %. Observations on germination phenology were conducted on the seedlings at daily intervals. To establish the optimum temperature for highest germination percentages seeds were germinated at different constant temperatures, 12°C, 15°C, 20°C, 25°C, 35°C, and 40°C. To test germination dependence on light condition the seeds were germinated in both in the dark or light conditions. Descriptive statistics including mean value, and standard deviation were calculated for every data set using Jandel Scientific statistical package. Mean, standard deviation, and minimum and maximum values were calculated for each quantitative data set. One-way ANOVA ($p< 0.05$) was used to test differences in environmental data, species cover, and canopy height of habitat types. Dunnett's method has been for honestly significant difference test was used to equal variances assumed. The principal components analyses (PCA) were based on the covariance matrix of the coefficients and not on the correlation matrix, because coefficients with small variance covariance values of harmonics of Fourier coefficients are generally not important for explaining the observed morphological variations of seeds shape. Stepwise discriminant function analysis (DFA) was used to determine which quantitative are more for discriminating among the studied taxa. The analysis was performed using the software packages SPSS v.16.0 for Windows (SPSS Inc., 2014).

Results

Germination Morphology - B

litwinowii has pistillate catkins disintegrating at time of seed dispersal, each scale is subtending 3 flowers (**Figure 1A**). Fruit is a samara with 2 lateral wings and tipped by 2 persistent styles. Ovary semicarpous, inferior with 2 anatropous ovules located on merged marginal placentas resembling a central placenta. Only one of these 2 ovules will give rise to the seed. The embryo starts to grow inside a fruit after imbibition. Some fruits with unfertilized ovules deprived of embryo start to enlarge after imbibition (**Figure 1B**). This enlargement proceeds due to proliferation of endocarp cells layers. Germination starts with appearance of a radicle emerging from the terminal slits of a pericarp located between styles (**Figure 1C**). Radicle turns at 180°C and grows towards the fruit base. Soon after emerging, root hairs appear at the surface of a radicle (**Figure 1D**). Cotyledons are green when appear. They first stay closed and unfold and enlarge later. *R. caucasicum* has long septicidal capsule dehiscing many seeds (**Figure 2A**) germinating outside of a fruit. We have been working on the microscope and ENM species have been finding in the photo (**Figure 2B**). These ENM species are in the *R. caucasicus* seed inside and we have found one species - *Oidiodendron maius*. Embryo is small with undifferentiated cotyledons. It starts to enlarge after imbibition (**Figure 2C**). Radicle emerges and grows but in difference to *B. litwinowii* it does not have root hairs (**Figure 2D**) until first leaves are developed.

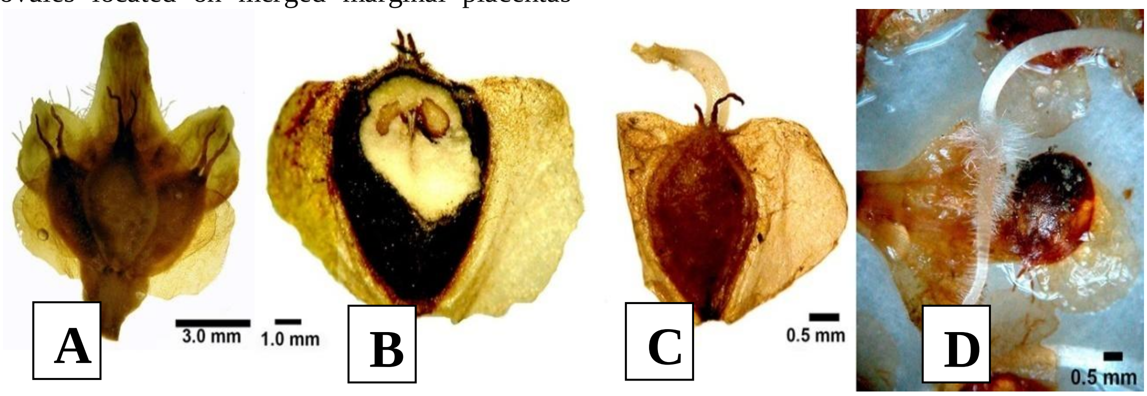


Figure1. *Seed germination and seedling development in B. litwinowii. A - Scale subtending 3 fruits. Samara with 2 lateral wings and 2 persistent styles; B - Fruit with unfertilized ovules deprived of embryo enlarged after imbibition due to endocarp proliferation. Semicarpus ovary with 2 anatropous ovules before fertilization located on merged marginal placentas resembling a central placenta; C - Radicle emergence growth of a seedling; D - Root hairs on the surface of a radicle.*

Germination Penology and Test

The developmental stages of seed germination and seedling development were defined as follows: imbibition, germination, growth and seedling. The duration of each phenological stage was determined as a mean duration observed for the individual seeds or seedlings (**Figure 3**). Imbibition in *B. litwinowii* needed 2 days and germination took place after 4 days. Growth stage was prolonged and took about 2 weeks. Different duration of the developmental stages was observed in *R. caucasicum*. Here, imbibition was longest period (ca. 3 weeks) and after germination seedling development was performed very rapidly. The germination test showed that percentages of imbibed seeds were higher in all treatments than germination percentages. The germination tests at different light conditions have shown that no germination has occurred in *R. caucasicum* in the dark (**Figure 4**). *B. litwinowii* was germinated in both treatments. The germination tests at different temperature revealed highest germination percentage at 25°C for both species (**Figure 5A,B**).

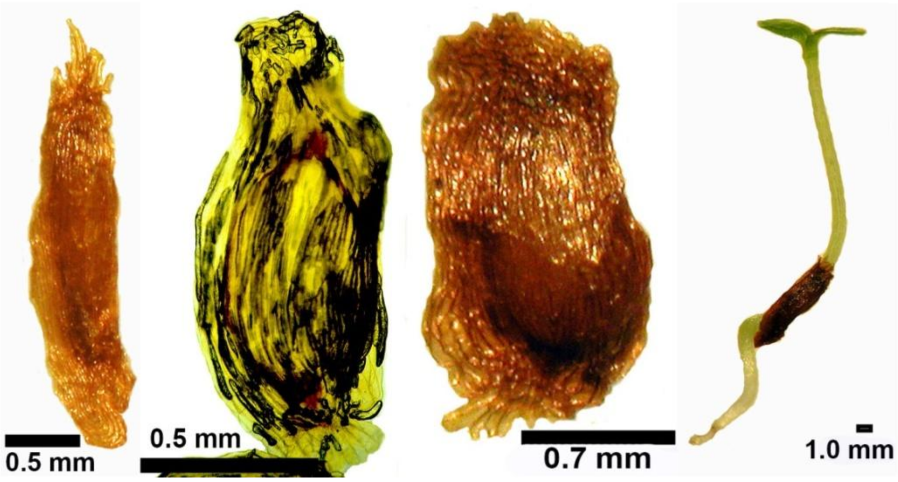


Figure2. *Seed germination and seedling development in R. caucasicum: **A** – Seed before imbibition; **B** – R. caucasicus seeds are in the inside with ENM one species - Oidiodendron maius and it activates to germination in alpine treeline and till 2900 m; **C** - Imbibed seed with growing embryo; **D** - Radicle emergence growth of the seedling. Appearance is open of the cotyledons.*

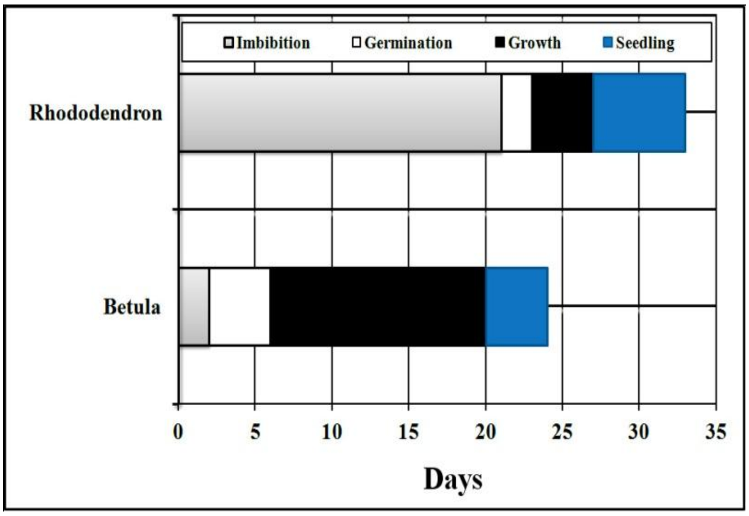


Figure3. *Mean duration of the developmental stages of seed germination and seedling development in B. litwinowii and R. caucasicum. n=500.*

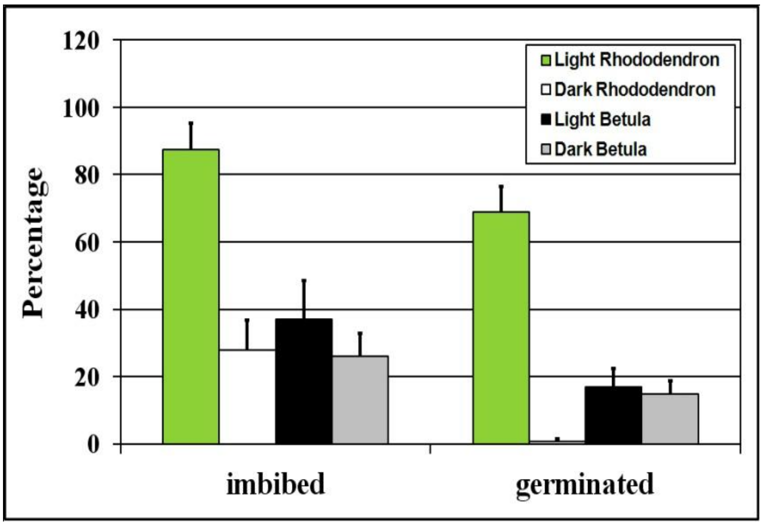


Figure4. *Percentages of imbibed and germinated seeds of B. litwinowii and R. caucasicum germinating in light and in the dark at similar temperature (25° C) in laboratory conditions. N=5.*

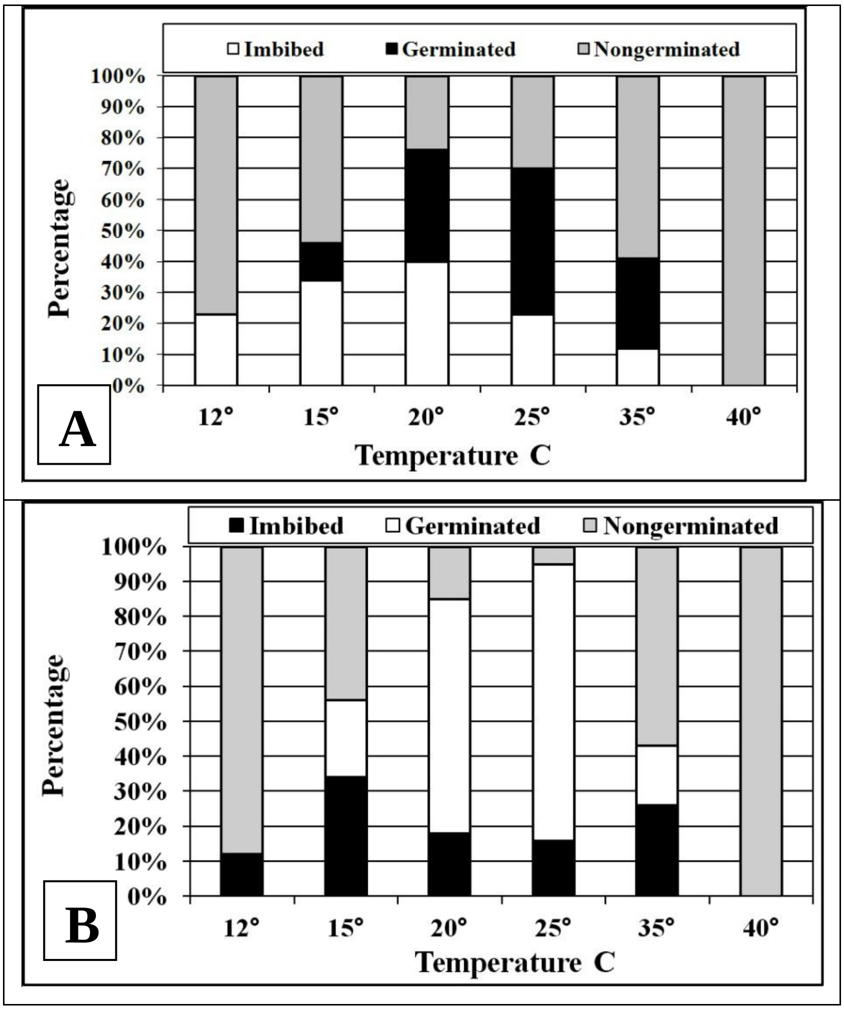


Figure5. *Percentages of imbibed, germinated and nongerminated seeds of B. litwinowii (A, left) and R. caucasicum (B, right) tested in laboratory conditions at different constant temperatures. n=5.*

Field Observations

Seeds harvested at highest elevation in the natural treeline - Site 5, have shown lowest germination percentage. Seedlings from 1-12 years-old were found at all study sites, ranging from a low mean abundance of 0.16 seedling s/m^2 at Site 1 (birch forest) to a high of 1.52/m^2 at the mid-elevation, timberline Site 2. The understory Sites 5 and 3 had the next greatest abundance (0.74 and 0.54 seedlings/m^2, respectively), followed by the more exposed Sites 5 and 3 (0.24 seedling s/m^2), and then Site 1 (0.14 seedling s/m^2). There was no statistical correlation between site elevation and seedling abundance (p = 0.01, ANOVA, **Table 3**). Seed bank analyses has shown that the number of seeds of birch, *Rhododendron* and other plant species varies significantly in study sites (**Figure 6A**). There was no statistical correlation between the number of seeds in the site and seedling abundance (r^2=0.2; p = 0.001, ANOVA). Results of the DFA has grouping variables are number of seeds in these sites and lower alpine timberline is with many seeds and treeline sites are in related to seeds points are as equal (**Table 3; Figure 6B**).

Table3. *Numbers of seeds in soil cover from 5 sites of Kazbegy are as percentage by understory vegetation: tree as B. litwinowii, shrub as R. caucasicus, and other trees and shrubs are in timberline and in treeline from 1850 to 2553 m. One-way analysis of variance (ANOVA) was conducted for all values. Discriminant function analysis (DFA) and F significance values are presented on stepwise. (N = 100).*

Characters	*Betula*	*Rhododendron*	Others
Eigenvalue	**21.897**	**0.138**	**0.078**
% of Variance	**99.0**	**0.6**	**0.4**
Cumulative %	**99.0**	**99.6**	**100.0**
Canonical Correlation	**0.978**	**0.348**	**0.268**
Levene Statistic	**33.267**	**6.886**	**5.980**

Wilks' Lambda	**0.074**	**0.879**	**0.438**
df1	**4**	**4**	**4**
df2	**78**	**78**	**78**
Mean Square	**7167708,064**	**1349,334**	**19326,529**
F	**245,311**	**2,691**	**25,038**
Sig.	**0,001**	**0,001**	**0,001**

Seed Germination in Mycorrhizael Soil

The seeds have been germinated in soils which coming from Kazbegian sites and seed germination is very active for mycorrhizal species. Collected in study sites are matured under different environmental conditions and some fungi are in sites (**Table 2**). Some sites have revealed various percentages of seed imbibition and germination (**Figure 7**). This activation of seed germination is for *B. litwinowii* with ECM mycorrhizal species. Site 1 from 2072 m has 14 species and very active were two species from these areas we made soil and seed germination was very active. These ECM species are *Boletus edulis* f. *tardus* (**Figure 8A**) and *Leccinum scabrum* (**Figure 8B**). Site 3 is on southern alternative of forest areas were *B. litwinowii* is lower height and we have found two ECM species - *Amanita muscaria* (**Figure 8C**) and *Amanita pantherina* (**Figure 8D**). Sites 2 and 4 High tall tree in timberline and treeline have more ECM species and three species are contacted with shrubs - *Russula aeruginea* (**Figure 9A**), *Russula lilacea* (**Figure 9B**) and *Suillus collinitus* is with shrubs (**Figure 9C**). Sites 5 in the upper side of treeline till 2555 m not had ECM species and *B. litwinowii* seed germination is with lichens which has a fungus (**Figure 9D**).

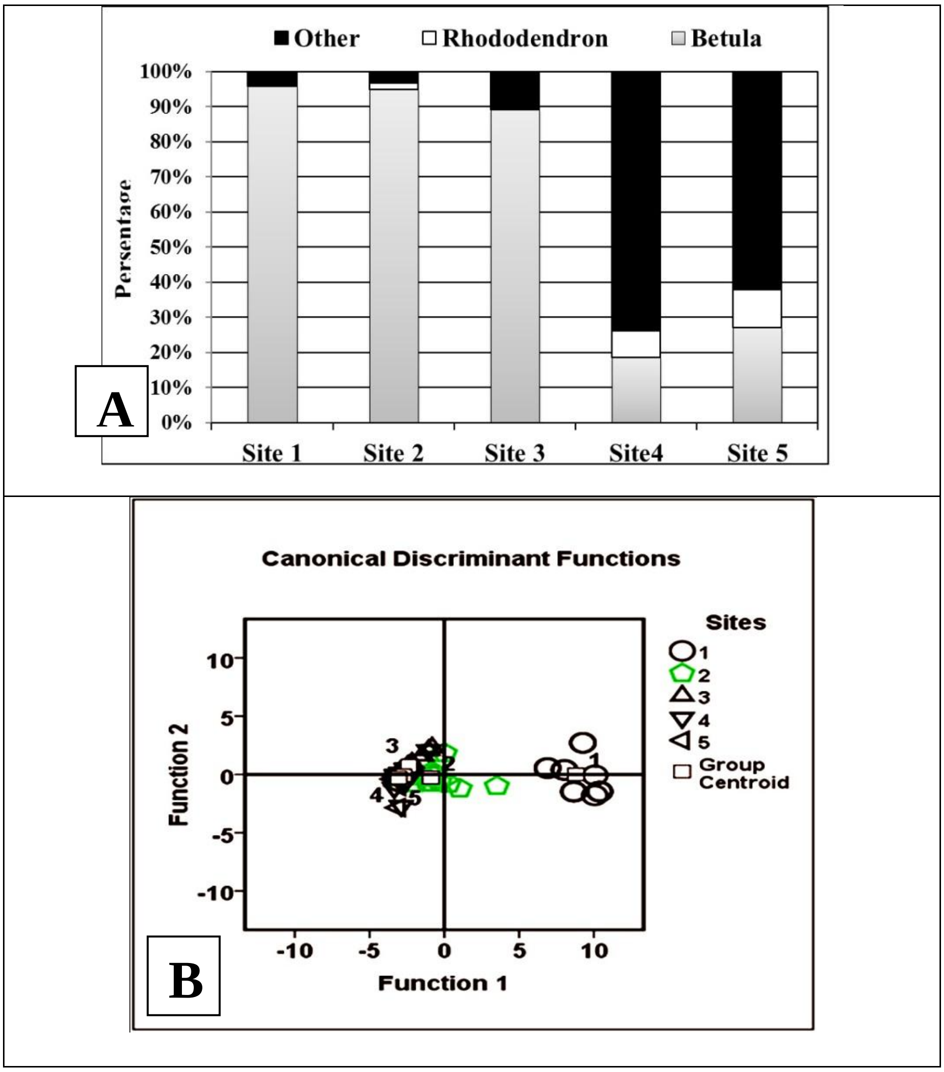


Figure 6(A). *Percentages of seeds of birch, Rhododendron and other species in soil samples collected in different study sites in August.* ***n=10. B*** *- DFA has number of seeds in 1-5 sites. Birch timberline in 1st Site is in different areas and 2-5 Sites are together with seeds.* ***n=500.***

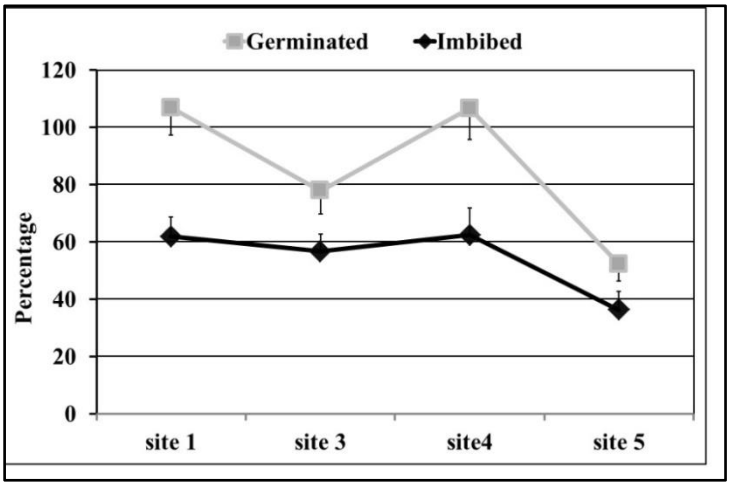


Figure7.*Percentages of imbibed and germinated seeds of B. litwinowii collected in different study sites and germinated in the laboratory under the same environmental conditions.* ***N=5.***

Figure 8

Figure 9

DISCUSSION

A limitation to the data presented here was the lack of comparisons between measurements for different seed germination study areas in this area. However, documentation does exist for the distinct vegetation patterns of this area, as well as the existence of the two distinct types of timberlines and treelines in this region of the Central Greater Caucasus Mountains of Georgia

[2]. These individual seeds/saplings become even more separated spatially as the distance from to the treeline increases. Seeds of *B. litwinowii* were already imbibed after 48 h and were germinated after 4 days. Although, cotyledon formation and seedling growth requires approximately 2 weeks. These data show that birch seedlings are more sensitive to drying and will not survive in open area under direct sunlight. Therefore, shaded and moist microhabitat under Rhododendron shrubs may facilitate successful establishment of birch seedlings at the alpine treeline. Radicle of the birch develops root hairs soon after emergence and is very sensitive to drying. Seedlings isolated from the Petri dish dry up already after 3 min.

Radicles of *R. caucasicum* have no root hairs until first leaf develops and seedlings are more resistant to drying. At the same time, seed imbibition was prolonged in *R. caucasicum* (3 weeks), but germination and cotyledon formation were happened very rapidly (2 days). Previous studies on *R. caucasicus* have focused on its competitive status with other commercially important forest tree species [14], although recent studies have shown little or no competitive interaction, as well as some evidence for a beneficial effect on tree seedlings [15]. One study has associated the ENM mycorrhizae of a *Rhododendron* understory with the successful growth and establishment of shrubs seedlings, attributed specifically to a lessening of incident sunlight and improved water relations [16]. This ecological ‘‘facilitation’’ in the germination and establishment process is in general agreement with the hypothesis that favorable microsites created by the presence of other plant species become increasingly important as the abiotic environment becomes more harsh [17]. Recent hypotheses of timberline causation have included the possibility that low soil temperatures may result from the large, forest-tree stature and the inherent shading of the soil surface with ECM mycorrhizael are in this areas [18] In contrast, the relatively high abundance of birch seedlings beneath the *R. caucasicum* canopy (Site 5), despite having the lowest minimum temperatures and the greatest number of cold days among all sites, seems to contradict any negative effects of cold soil temperatures. However, the polyhedral depressions of Site 5 and 3, where birch seedlings were found rarely, also had more constant soil temperatures (higher minimums and lower maximums) than the adjacent soil surfaces, implicating more constant soil temperatures as a possible influencing factor in successful seedling establishment (**Table 1**). In summary, the observation that these birch trees occur only on northern, lee slopes, where a deeper winter snowpack accumulates, may reflect the ecophysiological intolerance of this species to high sunlight exposure in such a cold environment, as measured for timberline conifer seedlings elsewhere [3]. Any disadvantage of colder air or soil temperatures to birch seedling establishment, hypothesized previously as an important factor regulating the maximum elevation of timberline and treelines appeared to be outweighed by the benefits of reduced sky exposure [19]. Also reported a similar impact on photosynthesis when shading from cold nighttime skies and daytime sunlight was combined, resulting in substantially lower photosynthesis than either alone.

The results of the study show clearly that there is much variation in the proportion of total resources allocated to reproduction among plants exposed to different treatments – partial shade, drought, moist and that growing under common environment [20]. The germination tests at different light conditions have shown that no germination has occurred in *R. caucasicum* in the dark. *B. litwinowii* was germinated in both treatments. We suppose that seed germination requirements of studied species determine their distribution patter in natural environment. *R. caucasicum* grows in better illuminated habitats under open subalpine krummholz and above treeline. But, it does not enter birch forest with closed canopy at lower elevations. However, there were no significant differences among treatments in the seed set characters within mycorrhizae [18]. The mean number of seeds matured per mycorrhizae was not significantly different in control and treated plants. mycorrhizae number per plant, rather than seed number per ECM and ENM was the component of resource limitation sensitive to lower light and water availability [6]. In many plant species, particularly in annual plants, which depend entirely on sexual reproduction by seed, large individuals produce much more seeds than small ones. A trade-off between vegetative growth and reproduction occurs when plant size varies as a function of the environment. Such a genetic similarity suggests that differences in plant size and fecundity in this highly plastic annual plant primarily reflects environmental factors and is unlikely to be the basis of evolutionary changes. On the other side, when the variation in size is due to genetic differences, reproduction may be similar in both large and small plants.

However, seed sizes and germination ability are increased under shade. Water deficit does significantly decrease the number of seeds and pods and increases the abortion rate of initially formed seedlings. Moreover, the number and sizes of mature seeds are smaller and their ability for germination is normal. There was no correlation among seed number in the soil, germination ability of seeds harvesting in different study sites and seedling abundance in the sites. This might be indicative that the number of produced seeds and their quality are not the main determinants to successful establishment of seedlings and maintenance of the plant community structure, but in all probability, ecological “facilitation” in the germination and establishment process created by the presence of other plant species and favorable microsites plays more important role in plant propagation and maintenance of community structure. Higher seed abortion and reduced seed vitality are the consequence.

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