

The biological basis of the endangered relict species from the Salicaceae family conservation in eastern Poland



In the years 2010-2016, studies on the biology and ecology of two willow species, *Salix lapponum* and *S. myrtilloides*, were conducted to determine the methods of their conservation. In the first stage, data on the resources and condition of the population of both species surveyed in Polesie Lubelskie were updated. It showed an 80% decrease in the number of *S. lapponum* stands in Polesie Lubelskie Region since the 1950s. The existence of 5 from 35 stands was confirmed in the study area, regarding the populations, 18 were considered as extinct and 11 were not confirmed in the earlier described locations. In case of *S. myrtilloides*, only 3 populations were confirmed. The remaining stands given in the literature (14 stands in Łęczyńsko-Włodawa Lakeland) no longer exist.



Salix lapponum L. (downy willow) is an inconspicuous shrub, reaching a height of 1 m up to 2 m. Downy willow is a perennial plant, a nanophanerophyte, characterized by dioecy. It is an entomophilous and melliferous species. *S. lapponum* is commonly found in subarctic and boreal peatlands of northern and north-eastern Europe as well as of western Siberia. Isolated populations are also encountered in some mountain ranges of Central and South Europe as well as in Scotland. In lowland areas in Poland *S. lapponum* occurs mainly on the eastern side of Vistula. Currently, its confirmed stations are located on Łęczyńsko-Włodawa Lakeland in the Polesie National Park, in the Biebrza National Park and in the Knyszyńska Forest. Two populations in Karkonosze Mountains grows on the subalpine floor as a endemic *Salicetum lapponum* association W. Mat. 1965. *S. lapponum* is legally protected in Poland and the number of its stands and population size have systematically decreased since the 1950's. As a glacial relict, it has the status of endangered species (CR) according to the 'Polish Red Book of Plants'. The species should be subjected to immediate conservation in order to restore adequate levels of distribution of the populations that still exist in Poland.

Salix myrtilloides L. (swamp willow) it is a deciduous small shrub growing to 15-60 cm tall, strongly related to raised and transitional peat bogs habitat. *S. myrtilloides* is an Euro-Siberian species. Its range covers Eastern and Central Europe as well as almost the whole of Siberia. The western limit of its range runs through Poland, in the regions of Sudety, Tatra and South Carpathian Mountains. Isolated stands of *S. myrtilloides* can be found in the Swiss Alps, in the mountains of southern Bavaria, and in the Don River valley. *S. myrtilloides* is considered in Poland as a glacial relict species. In the 1950's it was reported in about 90 localities, but most of these stands do not exist any longer. It has been under a full protection in Poland since 1983. It is included in the 'Red List of Plants and Fungi in Poland' as a critically endangered species (category of threat - E) and in the 'Polish Red Book of Plants' as an endangered species (category of threat - EN). The main threat to *S. myrtilloides* populations is indirect and direct human activity, habitat eutrophication, drainage of peat bogs, and expansion of other woody species.

Ecological and biological studies

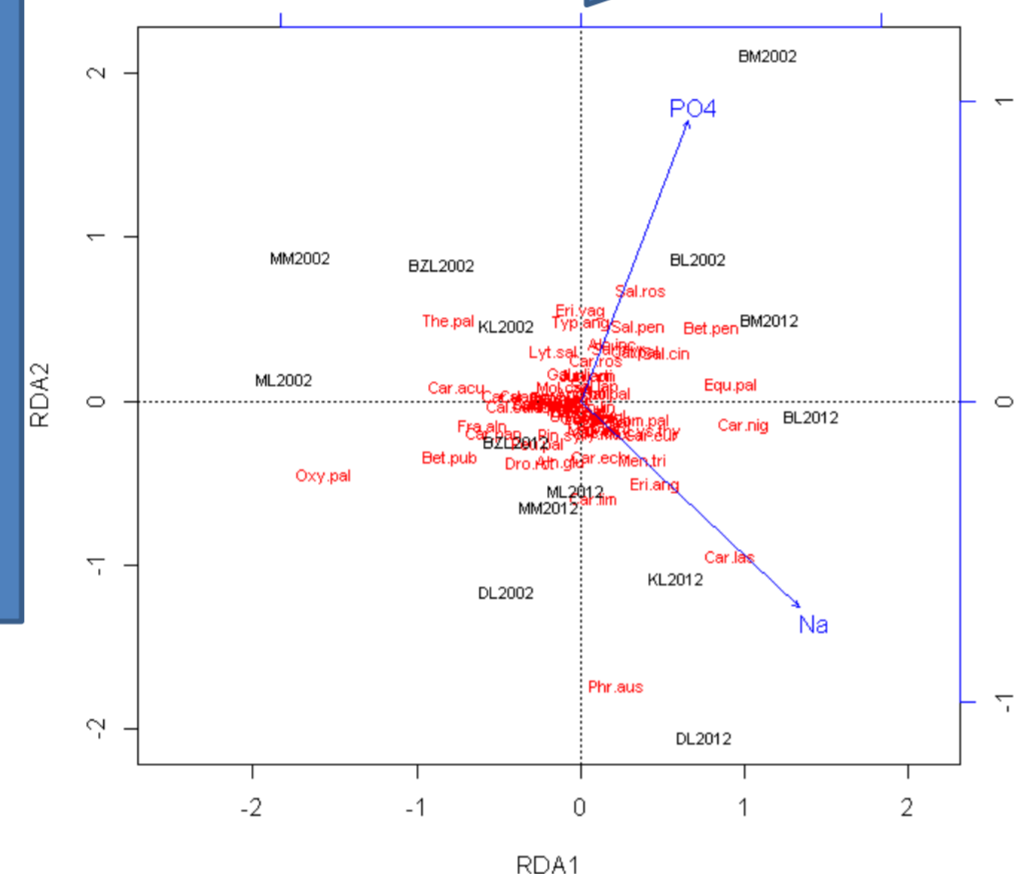
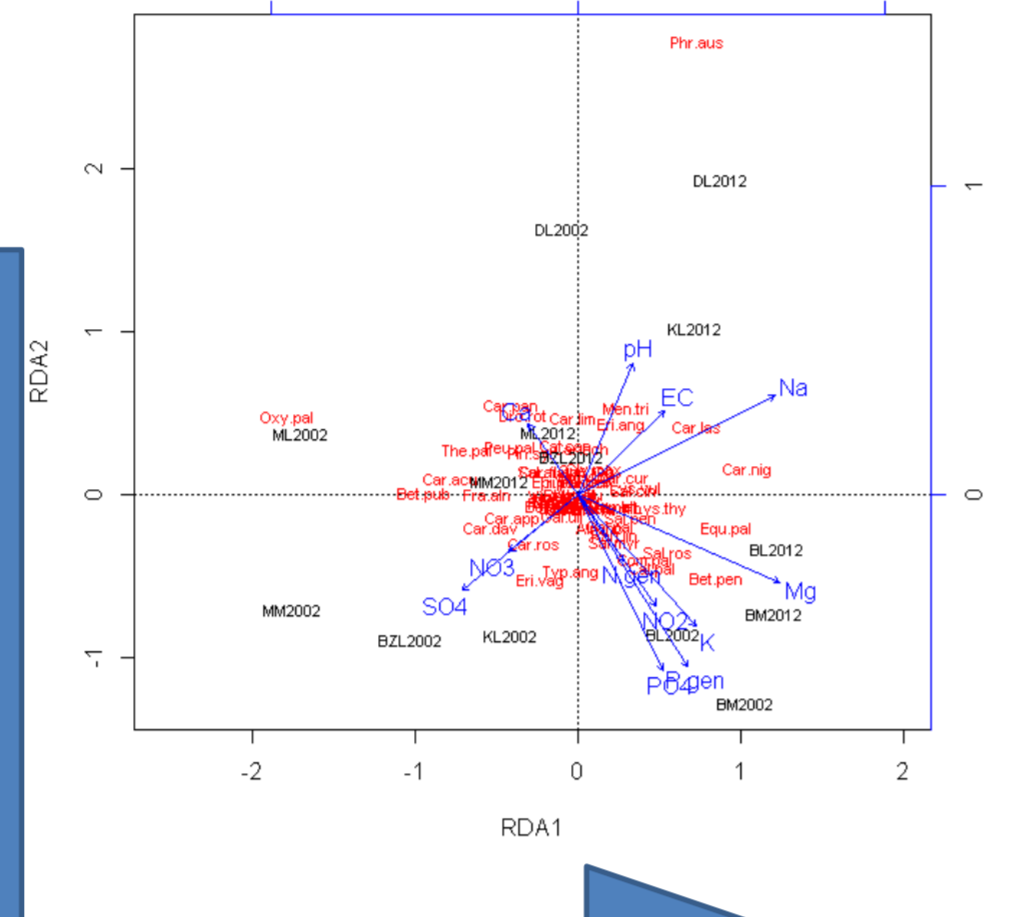
Habitat conditions

The study of habitat conditions, based on physical and chemical factors of groundwater analyzes and the composition of phytocoenoses in the stands, allowed to determine a set of conditions conducive for survival of the species.

The analysis showed that there was a strong correlation of concentration of Na ions in the water sampled from the investigated habitats with electrolytic conductivity and water pH as well as a negative correlation of Na, with NO_3 and SO_4 . Likewise, there was a strong positive correlation between water concentration of PO_4 , and Mg, NO_2 , N, P as well as a negative correlation for PO_4 and Ca.

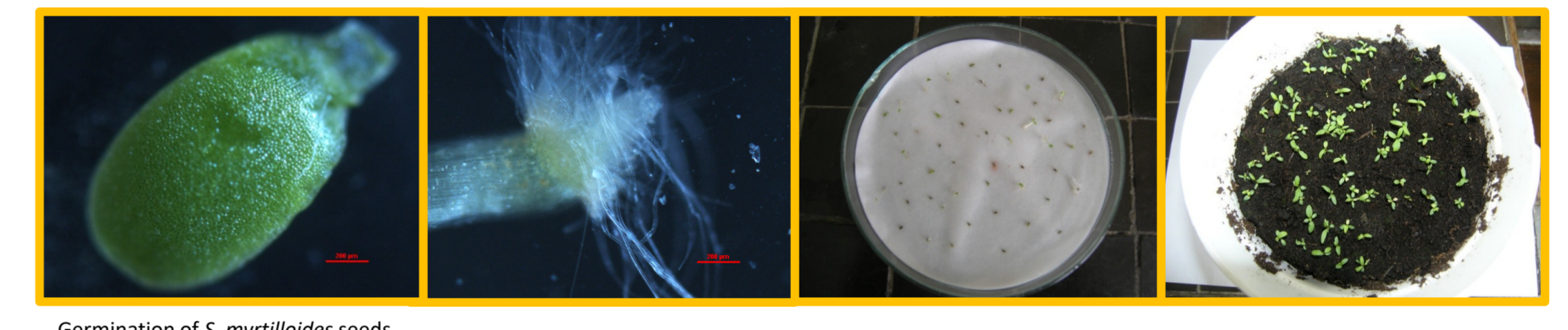
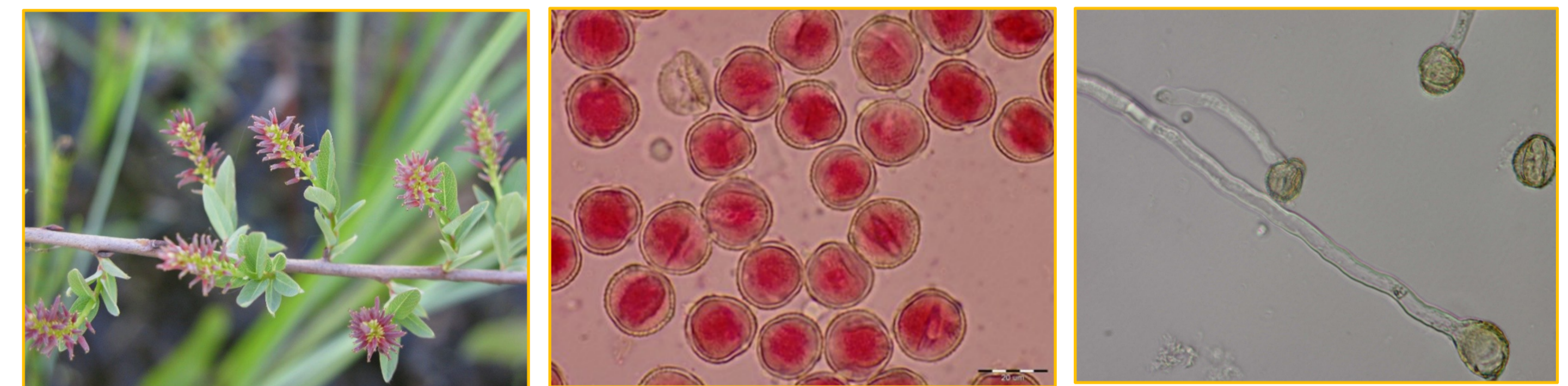
At the next stage of the analysis, stepwise selection of environmental variables was performed (using Akaike's information criterion as the selection criterion) and a statistical analysis was made of their effect on variations in the phytocoenoses. The stepwise selection of the variables shows that only Na and PO_4 are significant ($p < 0.05$) variables in the model. The other environmental variables are not statistically significant ($p > 0.05$).

Redundancy analysis (RDA) ordination diagram (showing the first and second ordination axes) displaying the relation of phytocoenoses and the correlation pattern of all environmental characteristics (arrows).



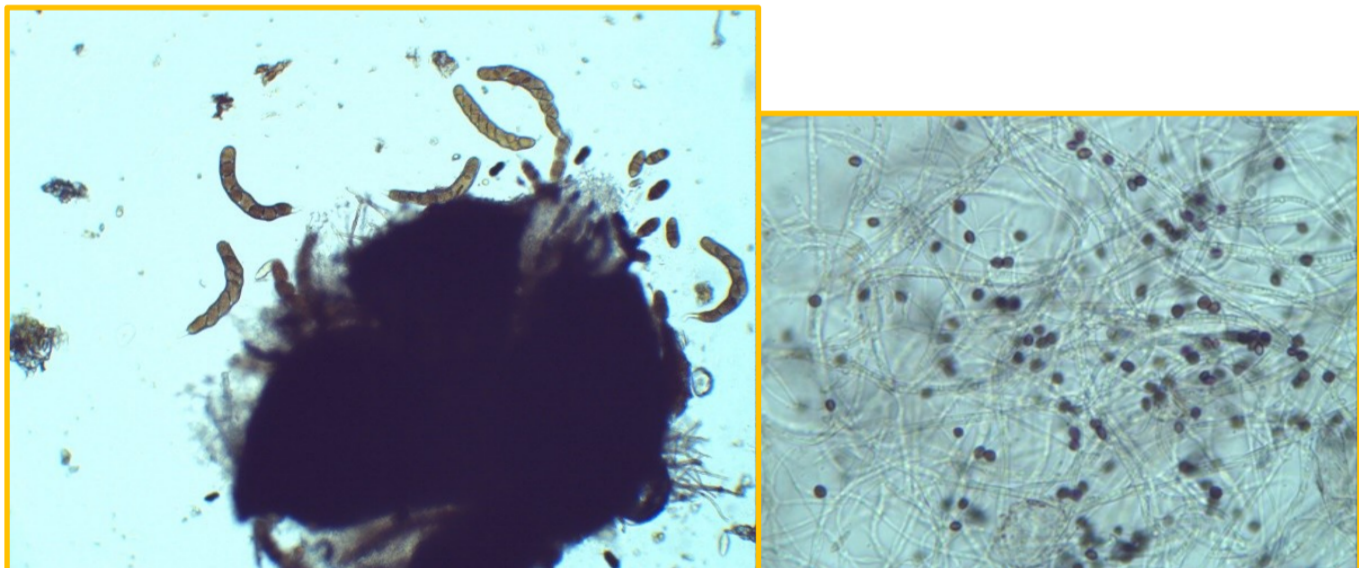
Reproduction and life cycles processes

It has been found that the predominant propagation of both *S. lapponum* and *S. myrtilloides* is the generative reproduction. It is demonstrated by the high pollen grains viability and germination ability, high germination capacity of seeds and the results of genetic analyzes confirming the absence of clones in the studied populations.



Entomological studies

phytophagous arthropods



Phytopathological research

pathogens

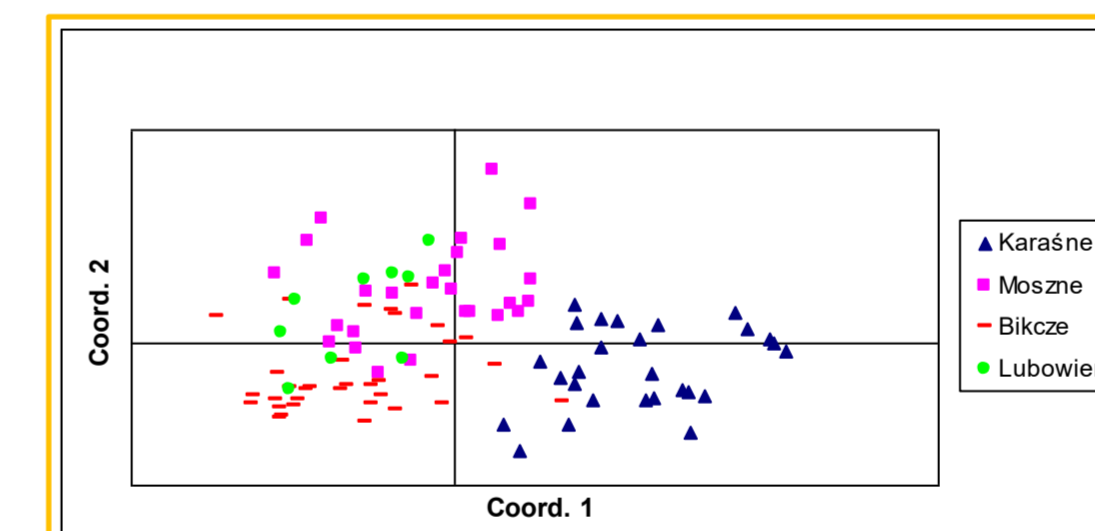


Reaction to stress

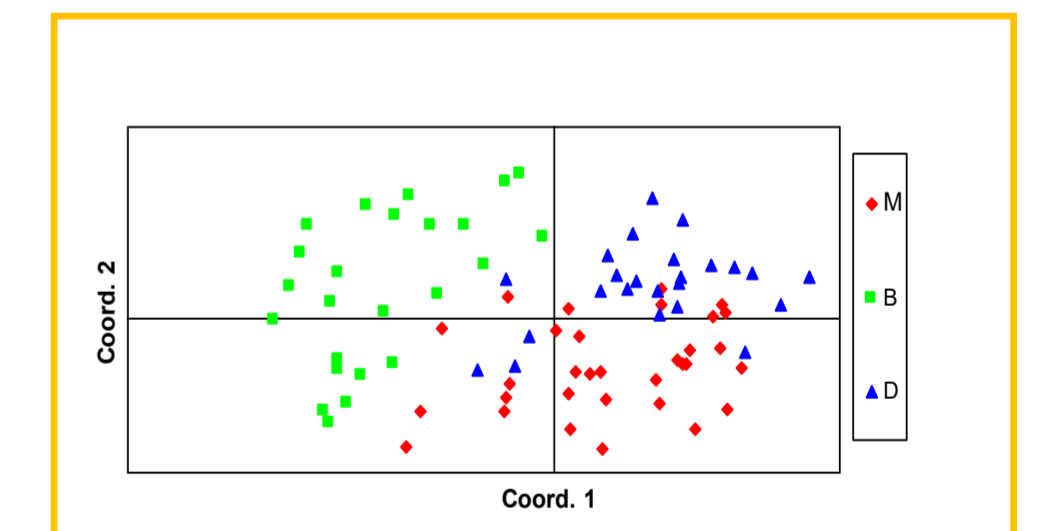
chlorophyll a fluorescence
histochemical detection of H_2O_2 in leaf blades tissues

Genetic diversity

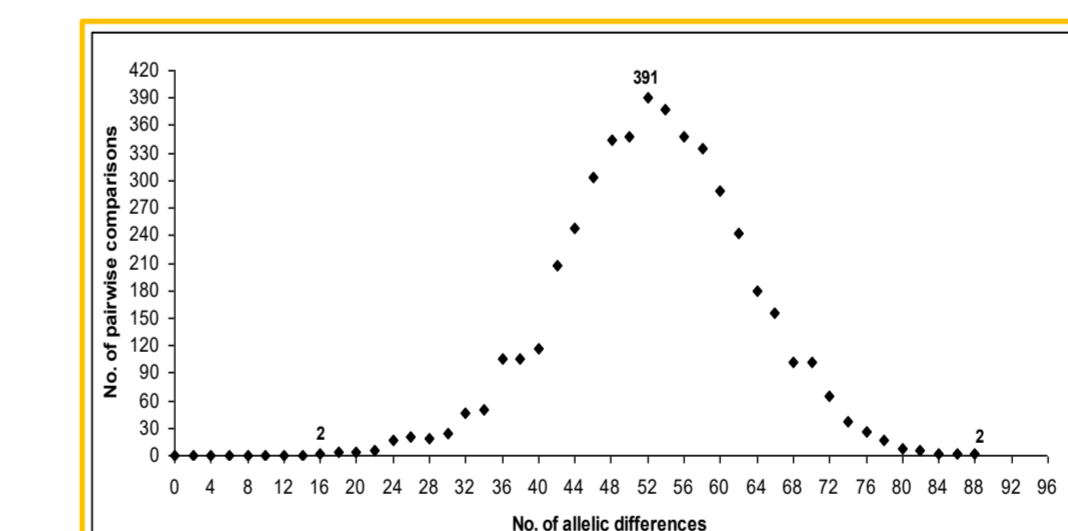
The genetic diversity between the populations of both *S. lapponum* and *S. myrtilloides* was not high, but statistically significant, indicating established differences in allele frequencies and lack of gene flow (ISSR analysis). This result is not surprising as, since the mid-twentieth, the changes in the environment were mainly caused by anthropogenic factors which resulted in habitat fragmentation and displacement of the species with narrow ecological tolerance. Despite this, the relatively high diversity within the population preserves the survival of the species in the study area.



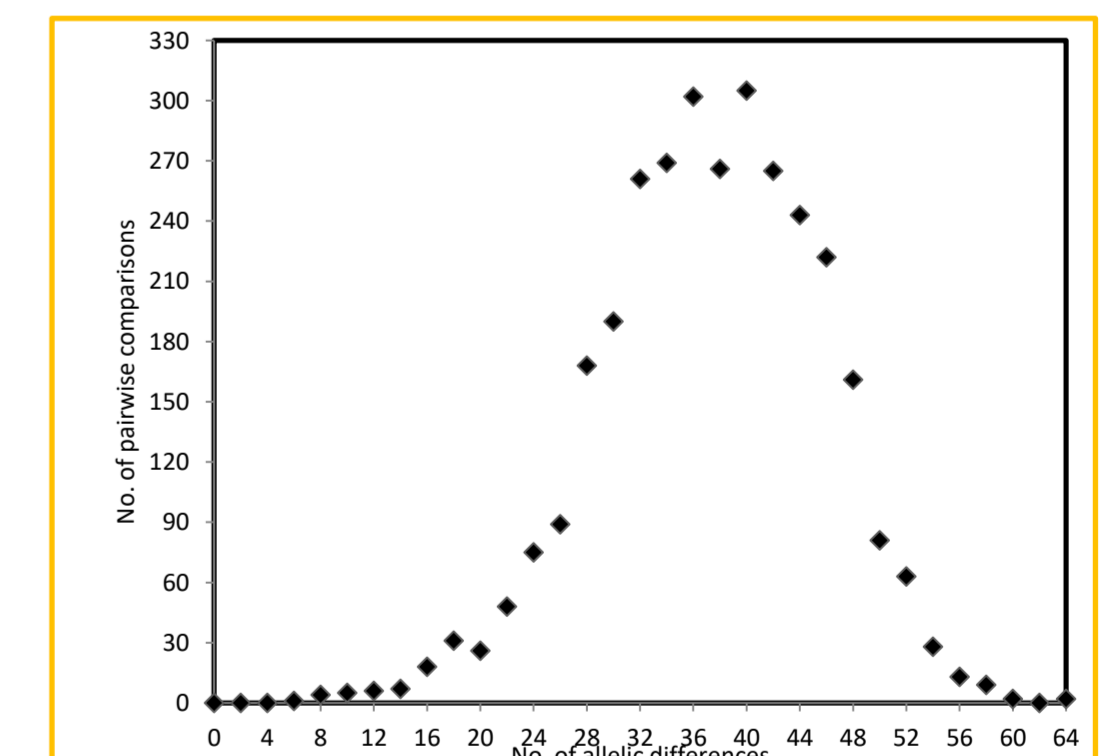
Principal Coordinate Analysis (PCoA) of 4 populations of *Salix lapponum*.



Principal Coordinate Analysis (PCoA) for 3 populations of *S. myrtilloides*.



A histogram of the number of allelic differences for pair-wise comparisons for *S. lapponum* individuals based on GenoType results.



A histogram of the number of allelic differences for pair-wise comparisons for *S. myrtilloides* based on GenoType software results.

Protection by reintroduction

It was decided that propagation *ex situ* and reintroduction of the obtained specimens would be the most effective methods of active conservation of the species. Due to a very low amount of plant material and unsuccessful germination of seeds, micropropagation was chosen as the only possible way to obtain young plants.

The acclimatization stage was conducted in high humidity conditions, temperature of 20°C and light intensity of $30 \mu\text{mol} \cdot \text{s}^{-1} \cdot \text{m}^{-2}$ at 16-hour photoperiod. More than 90% of plantlets survived acclimatization process.

The obtained plants were reintroduced into natural stands in the third decade of May 2017. At present, the plants are monitored to develop the methods of active protection of the Salicaceae species.

In vitro cultures of the endangered species might be a key stage of active conservation, thereby making it possible to preserve gene resources of *S. lapponum* and *S. myrtilloides* and other plants that are threatened with extinction.



Reintroduction of *Salix* on peat bog Bilizonki (Poleski National Park)



Micropropagation and acclimatization process

