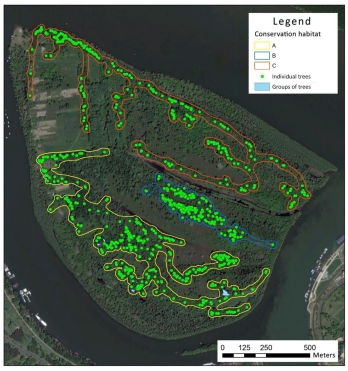

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In situ conservation of black poplar (*Populus nigra* L.) gene pool in the protected area "Great War Island"

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Abstract

The conducted research that involved the assessment of the census size of the black poplar population on The Great War Island, its viability and health status, levels of genetic diversity and recent changes in population served as the basis for defining the measures of *in situ* conservation of the available gene pool. A network of *in situ* conservation habitats, labeled A, B and C, were formed. The area of conservation habitat A is 27.90 ha and includes 455 individual trees of black poplar. Conservation habitat B spreads over an area of 7.84 ha and includes 192 individual trees of black poplar. Conservation site C spreads over an area of 21.25 ha and includes 260 individual trees of black poplar. Potential new areas suitable for natural regeneration have been identified in the vicinity of the conservation habitats. Their total area amounts to 16.50 ha and they are surrounded by reproductively mature black poplar trees and thus, seeds can easily be transferred to these areas. The suggested measures of *in situ* conservation are aimed at the maintenance and conservation of existing black poplar population in the area of Great War Island.

Keywords

Black poplar; Great War Island; Conservation; Conservation habitats; Natural regeneration

Contents

1 Introduction

Worldwide conservation of forest genetic resources aims at maintaining the overall genetic diversity of known or potential socio-economic or ecological importance (Mataruga et al. 2013). In addition, it is essential for the improvement and development of protective, aesthetic and cultural functions of forest ecosystems

(Šijačić-Nikolić and Milovanović 2007), including sustainable economic growth, development, and adaptation to the environment (Orlović et al. 2014).

Biodiversity reduction is a turning point and the best indicator of the global ecological crisis. Therefore, reduction of biodiversity can be listed as one of the basic problems of our time (Berberović 2012). In order to minimize the negative impacts of the growing loss of genetic diversity in forest ecosystems, there is a need for conservation and sustainable utilization of forest genetic resources, especially in the case of rare, relict, endemic and endangered species of forest trees. These species are considered high priority in the processes of conservation and sustainable utilization of forest genetic resources (Isajev and Šijačić-Nikolić 2001, 2003; Milovanović and Šijačić-Nikolić 2006, 2008, 2010).

According to the REFORGEN database on forest genetic resources (2003), *Populus nigra* L. is classified in the category of endangered species in the whole of Europe. The vulnerability of natural black poplar populations points to the necessity of developing a strategy for the conservation of its genetic diversity and implementation of conservation measures in its typical habitats, which was recognized by a number of scientists who have studied black poplar gene pool conservation Europe-wide (Cagelli and Lefevre 1995; Lefevre et al. 1998, 2001; Cottrell 2004; Storme et al. 2004; Kajba et al. 2005; Toplu 2005; Pospíšková and Bartáková 2004; Pospíšková and Šálková 2006; Ballian and Mekić 2008; Smulders et al. 2008; Rathmacher et al. 2010).

These studies are necessary due to the fact that black poplar populations in riparian forests of Europe have been constantly decreasing in the past decades. Natural black poplar habitats have been endangered in many European countries and especially in Western Europe. *Populus nigra* L. is one of the most endangered species in Belgium, where it has completely disappeared from the valley of the Meze River (van Slycken 1995), and a similar situation has been recognized in the Netherlands (de Vries 1995). A reduction of black poplar populations in Austria along the Danube, has been noticeable since 1960 (Heinze 1997). In Germany, *Populus nigra* L. is categorized as a "vulnerable" species and placed on the National Red List (BfN 2008). The situation in the UK shows that *Populus nigra* L. is on the verge of extinction in border areas of its range. The latest research shows that there are 7,000 black poplar trees in England, Wales and Northern Ireland, of which 600 are female individuals (Cooper et al. 2002; Preston et al. 2002), which means a drastically reduced effective population size (White 1993; Tabbush 1996). In addition, due to urbanization and poor management of natural resources, *Populus nigra* L. has become one of the most endangered species among natural populations in Turkey, where it used to have a wide range (Toplu 2005).

The share of autochthonous poplar forests accounts for only 0.5-1.0% of the total growing stock of Serbia, and therefore it can be considered a rare species (Banković et al. 2009). According to Kovačević et al. (2010), black poplar and white poplar in Serbia are among the dominant woody species of autocthonous biocenosis in alluvial areas, which are rare or even endangered, especially black poplar. Therefore, protection of extant autochthonous populations of white, and particularly black poplar, is of great importance.

The "Great War Island" is a river island found in a wide estuary of rivers Danube and Sava. It is a protected area which belongs to a vulnerable ecosystems of hydrophile forests. These ecosystems are characterized by the presence of plant and

animal species that are, to a greater or less extent, dependent on the presence of water. Given the importance of these ecosystems, as well as their vulnerability to current climate change, conservation and sustainable utilization of forest tree species, as their main pillars, are of special importance for their sustainability and stability (Maksimović et al. 2013; Šijačić-Nikolić et al. 2014). On The Great War Island, *Populus nigra* L. occurs in mixed stands belonging to the forest type of white and black poplar forest (*Populetumalbo-nigrae*) on a mosaic of different alluvial soils (Banković and Medarević 2009). The presence of *Populus nigra* L. at this location is of crucial importance for the conservation of sensitive hydrophile forest ecosystems. With a 5.4% (2,458.7 m^3) share in volume and 7.0% (54.2 m^3) share in volume increment, *Populus nigra* L. belongs to the group of rare and endangered species in this area, which imposes the need for its remaining gene pool conservation and sustainable utilization.

The aim of our study is to assess census size of the black poplar population on The Great War Island, to delineate areas occupied by black poplar individuals, to assess viability, levels of genetic diversity and recent changes in population size in order to provide guidelines for population conservation.

2 Material and methods

Populus nigra population on The Great War Island may be considered as an isolated population because of the absence of populations of this species in its vicinity.

In 2011, we georeferenced all black poplar individuals present on The Great War Island, and carried out reconnaissance of the terrain, assessed the quality of trees and their health status (infestation by pests), and recorded the presence/absence of natural regeneration (Maksimović et al. 2016).

In our previous work, we assessed levels of genetic diversity in study population by analyzing 30 test trees with nuclear microsatellites (Maksimović et al. 2016). We used this data to assess recent size reductions (i.e. in the last 2 *Ne*–4 *Ne* generations) by performing the heterozygosity excess test of Cornuet and Luikart (1996) in BOTTLENECK 1.2.02 (Piry et al. 1999). The heterozygosity excess test was carried out assuming the IAM, SMM and TPM models (30% of IAM and 70% of SMM) of microsatellite evolution. Statistical significance was assessed using the one-tailed Wilcoxon test upon performing 10,000 replicates.

Generated data and the available knowledge of bioecological characteristics, silviculture and forest management of black poplar served as the basis for defining the measures of *in situ* conservation of the available black poplar gene pool in the area of The Great War Island aimed at conservation and sustainable utilization of the existing gene pool, which is a strategic approach to the conservation and enhancement of vitality of black poplar populations in the area of The Great War Island.

3 Results

In the area of The Great War Island, black poplar is present in fragments, with only 907 recorded trees (Figure 1), which are of good quality and are healthy. However, natural regeneration is lacking mainly because potentially suitable sites for establishment of seedlings are occupied by invasive species (Maksimović et al. 2014; Maksimović et al. 2016).

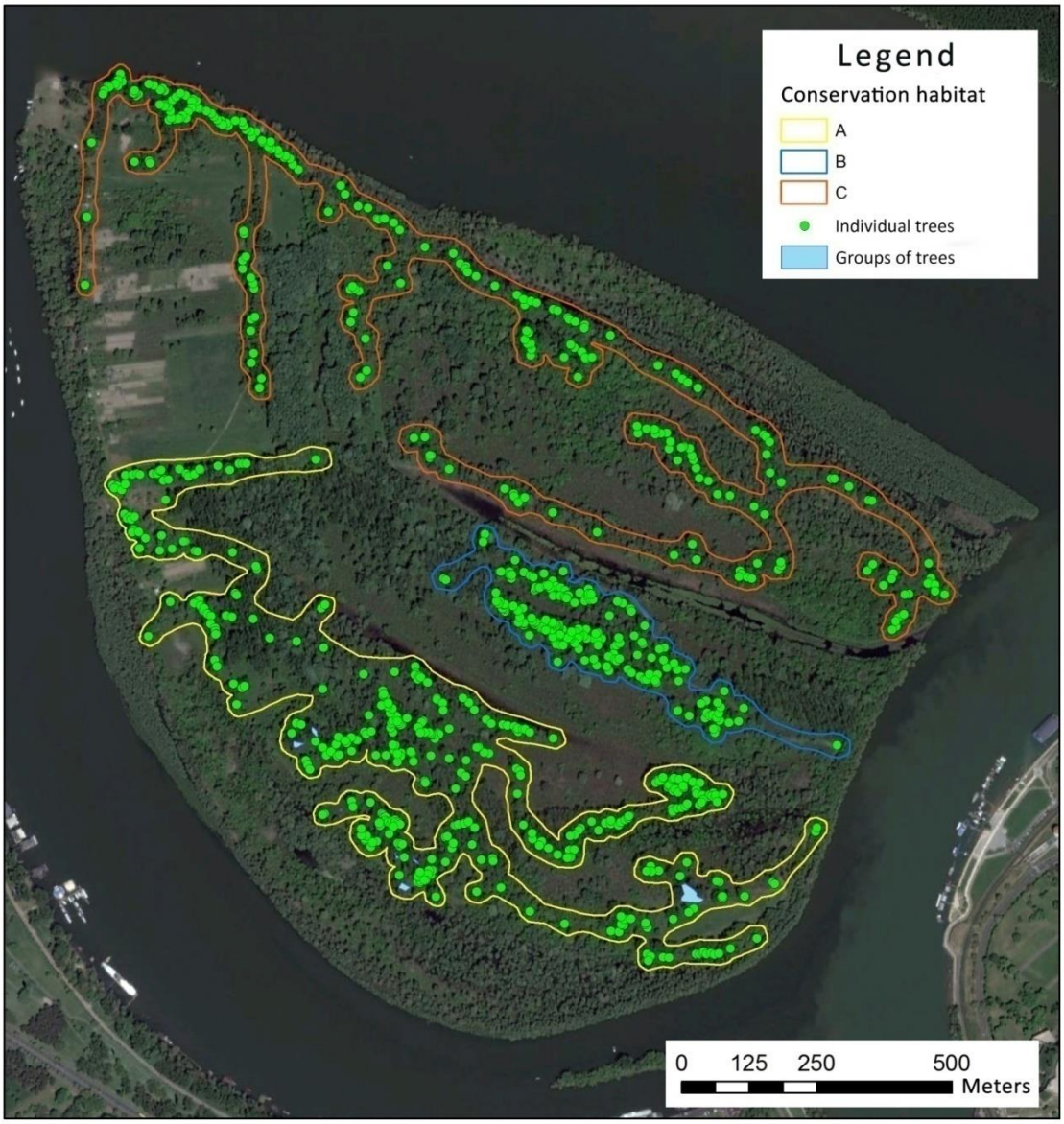

Figure 1. The network of *in situ* conservation habitats of black poplar (*Populus nigra* L.) in the area of Great War Island.

The levels of genetic diversity in black poplar population on The Great War Island is high (He = 0.822, SE = 0.025), with the fixation index (Fis) of 0.143 (SE = 0.045) (Maksimović et al. 2014).

Recent bottleneck (i.e. in the last 2 *Ne*–4 *Ne* generations) was inferred assuming IAM (P = 0.0002) and TPM (P = 0.0081) models, while according to the SMM model (P = 0.4829), population did not suffer from the recent size reduction. Since the TPM model is the model which best suits evolution of microsatellites in most cases, we inferred recent bottleneck in the studied population, which indicates the need for its remaining gene pool conservation.

The above mentioned activities carried out in the population of black poplar in the area of The Great War Island served as the basis for defining the measures for *in situ* conservation. Up to date, a network of *in situ* conservation sites was established (Figure 1). It includes the best trees and best-preserved stands of black poplar. In the

aim of facilitating planning and implementation measures of *in situ* conservation, a total of three conservation habitats were distinguished and given the letter codes A, B and C. Defined conservation habitats are spatially separated.

The area of conservation habitat A is 27.90 ha, including 455 single trees of black poplar. Looking downstream the river Danube, this conservation habitat is on the right side borders with a forest of white willow that grow on the banks of the Danube bayou, while the left side borders with a meadow in the central part of the island. Conservation habitat B spreads over an area of 7.84 ha and includes 192 individual trees of black poplar. Looking downstream the river Danube, conservation habitat B is on the right side borders with a meadow in the central part of the island, while his left side borders with Galijaš Canal. Conservation habitat C spreads over an area of 21.25 ha and includes 260 individual trees of black poplar. Looking downstream the river Danube, the right side from this conservation habitat borders with Galijaš Canal, while the left side borders with a forest of white willow on the banks of the Danube.

A network of *in situ* conservation habitats is a representative display of black poplar genetic resources in this protected area. All the conservation habitats are marked and visible, and in the coming period they should be marked with boards highlighting information on their significance for environmental protection and biodiversity conservation. In addition, in the aim of general health improvement in the conservation habitats, it is necessary to carry out thinning of silvicultural and sanitary character aimed at the removal of sick, dry and semi-dry trees, design new and maintain the existing roads and footpaths, conduct a detailed study of parasitic and saprophytic microflora, pay annual visits to conservation habitats, monitor them and organize adequate forest guard service.

New potential areas suitable for natural regeneration have been identified near the conservation habitats. Their total area is 16.50 ha and they are surrounded by reproductively mature trees of black poplar and thus, seeds can easily be dispersed on these areas. The mapping of potential areas for natural black poplar regeneration in the area of The Great War Island is shown in Figure 2.

Potential areas for natural regeneration are natural black poplar habitats, which are due to the absence of human intervention densely vegetated by false indigo-bush and other invasive woody and shrub species. In the aim of preparation these surfaces for the function of natural regeneration, it is necessary to remove undesirable shrub and weed vegetation and carry out deep plowing to destroy its root system.

Identification of potential areas for natural regeneration and removal of undesirable vegetation does not insure the process of natural regeneration, as black poplar regeneration requires specific conditions. By these specific conditions it is implied that at the time of seed maturity, the areas for regeneration have ideal conditions for natural regeneration, including bare land with fresh layers of sand and gravel, well-lit locations and a high level of groundwater. In addition to this, it is necessary for the seedlings to be well-rooted and stabilized in areas of regeneration, before encountering a new water wave to destroy the young plantlets. The maintenance of these areas is a rather expensive operation, and the effect of natural regeneration is uncertain. Therefore, conservation of genetic resources should be ensured by *ex situ* methods establishing clonal archive in the nursery and field gene bank in the area of The Great War Island of trees from the population.

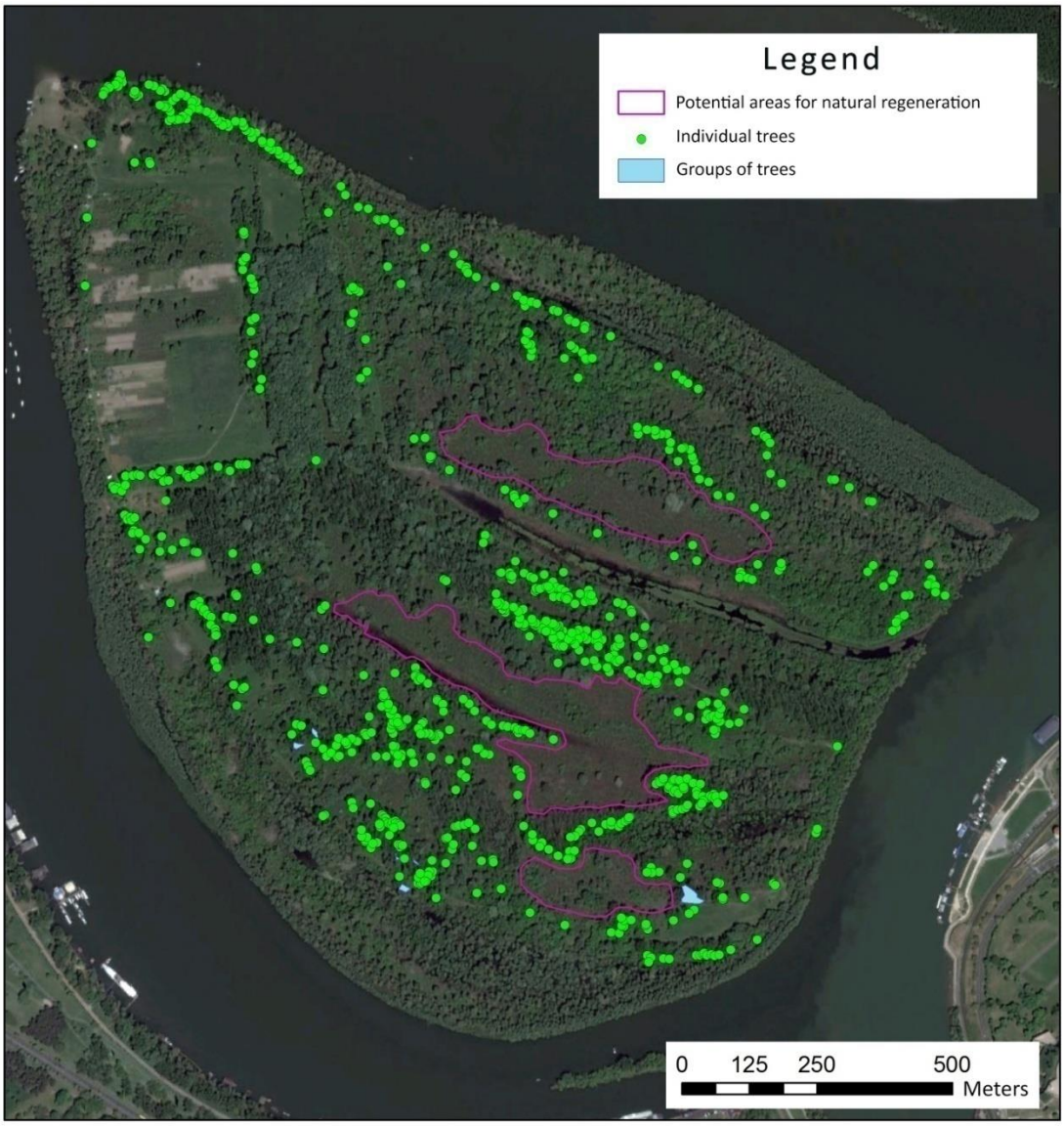

Figure 2. A map of potential areas for natural black poplar regeneration in Great War Island

4 Discussion

The basic preconditions for a successful conservation of forest genetic resources are stability and high levels of genetic variability in natural populations of forest trees (Pospíšková and Šálková 2006; Rathmacher et al. 2010; Jelić et al. 2014). Flush et al. (2002) suggests that in addition to that, the strategy for conservation of available gene pools should be based on an assessment of the population status. In addition, the decision for an appropriate conservation strategy has to been made on the basis of the species distribution, specific conservation objectives, bioecological characteristics of the species to be conserved, and the available knowledge of the silviculture and management of this species (FAO 1989; Šijačić-Nikolić and Milovanović 2010). In the case of the black poplar population on The Great War Island, we found

high levels of genetic diversity (He = 0.822, SE = 0.025) and signs of inbreeding (Fis = 0.143, SE = 0.045) (Maksimović et al. 2014). Furthermore, we found that this population experienced recent bottleneck (i.e. in the last 2 *Ne*–4 *Ne* generations). On the other hand, all individuals are healthy and of good quality, but natural regeneration is lacking. These findings altogether reveal that urgent conservation actions are required.

Facilitating natural regeneration on the black poplar population on The Great War Island is not an easy task because of the specific conditions required for seed germination and the establishment of new young populations of black poplar (Cottrell 2004; Pospíšková and Šálková 2006), which include well-lit sites with fresh drifts of sand and gravel and no vegetation (Barsoum 2001). As there are no such areas on The Great War Island, new potential areas for natural regeneration have been identified that should be adapted for this purpose by human intervention. Regeneration areas are located in the immediate vicinity of reproductively mature trees that surround them. Given the distance between the potential areas for natural regeneration and mature reproductive trees, seeds can easily be transferred to them by wind. This is confirmed by the results of research which dealt with determining the distance of pollen and seed dispersion (DiFazio 2004; Imbert and Lefèvre 2003; Pospíšková and Šálková 2006; Rathmacher et al. 2010; Bialozyt 2012). Most of these studies reveal a significantly longer transmission distance for pollen than for seed, and the main reason quoted for that is its size and weight compared to seed. Imbert and Lefèvre (2003) investigated the gene flow of black poplar along the Drome River in France and concluded that gene flow between populations exclusively occurs via pollen dispersion, while effective seed dispersion is limited to distances greater than 1-3 km. In their research, Rathmacher et al.(2010) found that the total effective transmission distance of pollen varied from 5 to 8,200 m, with an average value of 582 m. Most of the gene flow (i.e. 70%) took place at a distance of less than 1 km, whereas only about 4% of reproductively effective pollen was transmitted to distances farther than 2 km. The dispersion of seeds ranged from 23 to 6923 m, with only about 1% of seeds exceeding distance greater than 2 km. In addition, DiFazio (2004) noted a longer dispersion distance for pollen (440 m) than for seed transmission (220 m). On the other hand, in their research Pospíšková and Šálková (2006) note that the dispersion of pollen ranged from 10 to 230 m, whereas the dispersion of seeds from 163 to 370 m. Bialozyt (2012) noted that the largest percentage of pollen (75%) and seeds (86%) are dispersed at a distance of less than 1 km, while only a small percentage exceeds 2 km.

The results of the investigation of black poplar population in the area of The Great War Island indicate that effective genetic conservation can only be achieved through a combination of *in situ* and *ex situ* conservation measures, which is consistent with the findings of most researchers who have studied this issue. In this regard, Lefèvre et al. (2001) suggest that for the conservation of black poplar in natural populations, it is necessary to conduct a combined (*in situ* and *ex situ*) genetic conservation that should be integrated with intensive activities on the silviculture, conservation and restoration of habitats. Toplu (2005) stated that the programs of black poplar conservation in Turkey that started in the framework of the EUFORGEN network focus on *in situ* conservation measures. However, areas suitable for *in situ* conservation are limited, due to the vulnerability of natural populations to urbanization and poor management of rural resources, which often results in the

replacement of *in situ* by *ex situ* conservation, which has lately gained more attention. The strategy of forest genetic resources conservation of black poplar was divided into three operational objectives by Kajba et al. (2005), including: provision of the optimum possible amount of natural regeneration (appearance of malates), prevention of the loss of individual survivability in succeeding generations and identification and preservation of local and regional seed bases. In order to achieve these objectives, it is necessary to intensify the selection, breeding and testing of black poplar clones to get good quality planting material for the regeneration of riparian forests. Jelić et al. (2014) reported that for the preservation of black poplar populations, it is most suitable to implement its protection and enhancement of its natural habitats. However, *in situ* conservation will not be applied in large parts of its natural range along the Danube, due to urbanization and very frequent fragmentation and disturbance of natural stands. Therefore, *in situ* conservation may be limited to small areas, such as riparian forests isolated from cultivated plantations. On the other hand, Rathmacher et al. (2010) suggests that conservation should focus on large and diverse populations, in which it is necessary to provide new areas suitable for natural regeneration within the pollen and seed dispersion distance of potential parent trees. Conservation strategy should focus on the maintenance and improvement of metapopulations in close proximity, at a distance no greater than 1 km, where the largest part of the effective pollen is dispersed.

4 Conclusion

The outcomes of our study highlight the need for urgent actions required for the conservation of the black poplar population in the area of The Great War Island.

For the implementation of the *in situ* conservation measures, three *in situ* conservation habitats were defined on a total area of 56.99 ha (conservation site A with an area of 27.90 ha; conservation site B with an area of 7.84 ha and conservation site C with an area of 21.25 ha). New potential areas suitable for natural regeneration, which require costly operations for terrain modification, were identified on a total area of 16.50 ha. *Ex situ* conservation measures, which encompass establishing clonal archive in the nursery and field gene bank, have been recommended.

Suggested *in situ* and *ex situ* conservation measures will enable persistence of the black poplar population in the area of The Great War Island.

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