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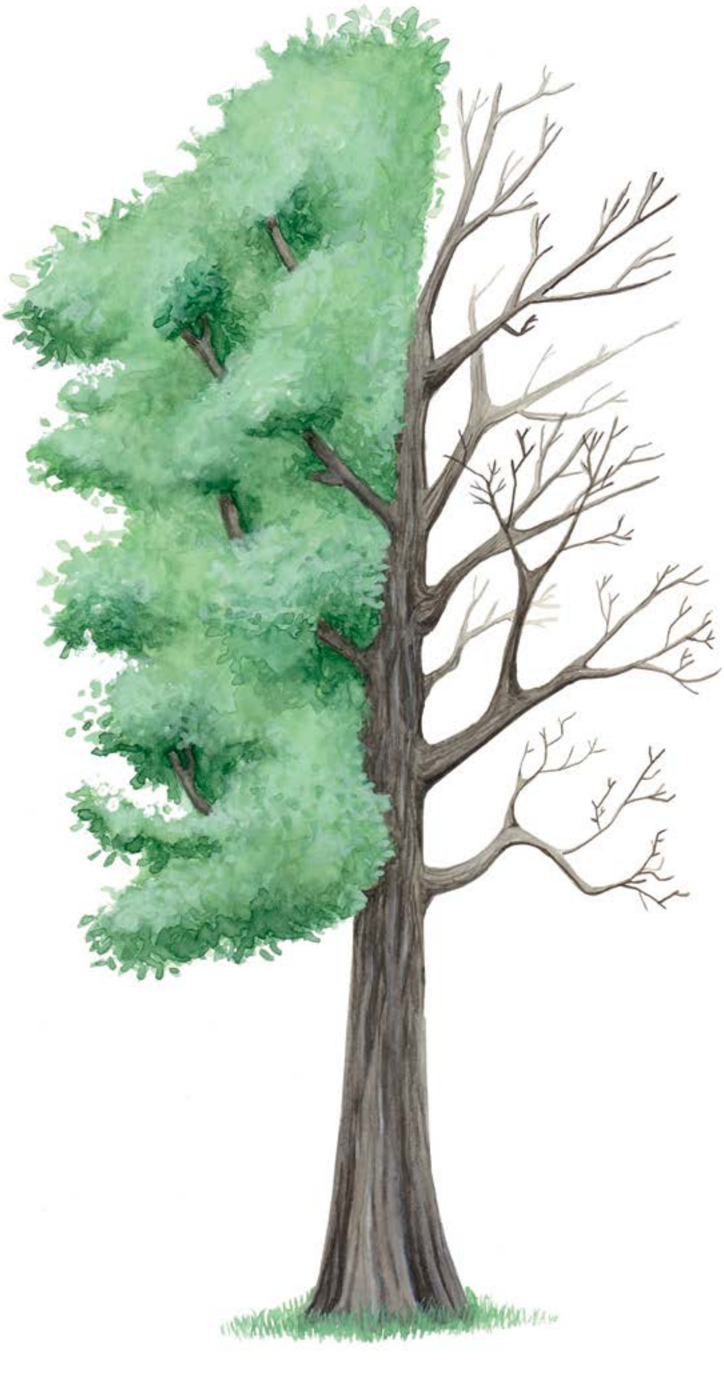
European black poplar
(*Populus nigra* L.)



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Guidelines for genetic monitoring of

9.2.5 European black poplar (*Populus nigra* L.)

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1 Executive summary

European black poplar (*Populus nigra* L.) is an ecologically important fast growing and short-lived deciduous forest tree species of mixed riparian forests with the physiological adaptation to colonise open areas after disturbances and survive changes associated with dynamic river systems. It enables natural control of flooding, is a keystone species for dynamic conservation and habitat restoration of floodplain forests, and is considered as an indicator species for the health and biodiversity of riparian ecosystems [1]. European black poplar is also known for its inherent ability to grow rapidly and taking up large volumes of water and nutrients from soil. This capability makes it important for phytoremediation, restoration, and environmental applications in polluted industrial zones, for microclimate regulation and for the improvement of biological diversity in open agricultural landscapes [2]. It is used as a parent pool for several poplar breeding programs around the world. It can be managed easily by coppicing, which makes it suitable for long-term conservation of the best genotypes of pure European black poplar plant material in *ex situ* collections. A wide range of recommendations for *in situ* conservation units and *ex situ* conservation methods were proposed within the framework of EUFORGEN [3] and later approved by several regional projects [4].

European black poplar naturally forms metapopulations of inter-linked local populations rather than small, isolated populations [6]. To ensure representative sampling across the metapopulation it is important to design a genetic monitoring system with randomly selected monitoring plots of adult trees in local populations, and monitoring plots in their natural regeneration centres along a river system as part of a complete network of interlinked local populations. Genetic identification of European black poplar trees must be performed by the use of species diagnostic DNA markers. The main obstacle to forest genetic monitoring (FGM) of European black poplar is finding habitats where the species reproduces effectively, and where conditions support long-term survival of the offspring.

These guidelines briefly describe European black poplar, its reproduction, environment, and threats, and provide guidance on establishing genetic monitoring plots *in situ* and recording all field level verifiers and background information.

2 Species description

The European black poplar (Figure 1) is a native, heliophilous and nutrient demanding deciduous forest tree species of temperate regions of Eurasia. It belongs to section *Aigeros* of the genus *Populus*, family *Salicaceae* [5]. It colonises open areas after disturbances, particularly due to dynamic river systems, and is found in the early successional stages of riparian mixed forest ecosystems. It forms different types of populations, that range from isolated trees to large pure or mixed stands. European black poplar naturally forms metapopulations comprised of smaller local populations [6, 7].

European black poplar is a medium to large-sized tree, generally reaching up to 40 m in height and up to 300 cm in diameter and living 100-200 years. In rare cases, individuals can reach 400 years of age [8, 9]. It often produces an irregular, branchy crown. The often crooked or swept, buttressed bole can be massive, frequently producing large burls or epicormic branches, but some trees in stands can also be straight and well formed [10]. The bark on mature trees is dark brown or black (Figure 2a) with numerous deep fissures [11]. Leaves are diamond-shaped to triangular, 5–12 cm long and 4–10 cm broad with the petiole of 2-6 cm in length [12, 13] with serrated margins and green on both surfaces (Figure 2b). Trees reach reproductive maturity within 10 to 15 years [14].

The morphological and phenological traits of *Populus nigra* can be used as a first level approach for characterisation of pure (not hybridised) European black poplar trees, at least in the case of adult and middle-aged individuals. The most stable species-specific traits and characters are detailed in the EUFORGEN identification sheet of *Populus nigra* [24]:

- shape of trees,
- epicormic shoots and dormant buds along the trunk
- intercrossing bark fissures along lower part of the trunk,

- leaf shapes (diamond, rhomboid or triangular),
- absence of European mistletoe (*Viscum album* L.) within the crown,
- presence of gall-making aphid species of the genus *Pemphigus* on leaves' petiole.

Based on experiences of European projects (EUROPOP, DANUBEPARKS, etc.) the trees, which were characterised by the above listed morphological traits and selected for gene conservation purposes, were in most cases also confirmed as 'pure' *Populus nigra* by diagnostic molecular markers.

(a) (b)

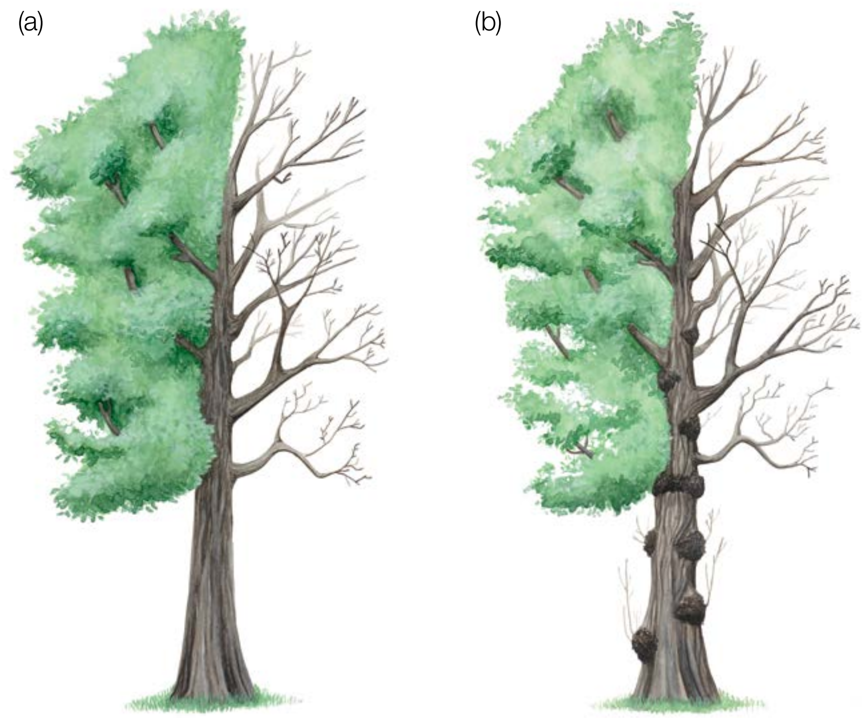

Figure 1: European black poplar (*Populus nigra*) habitus without epicormic shoots (a) and with epicormic shoots, which are a common feature (b).

(a) (b)

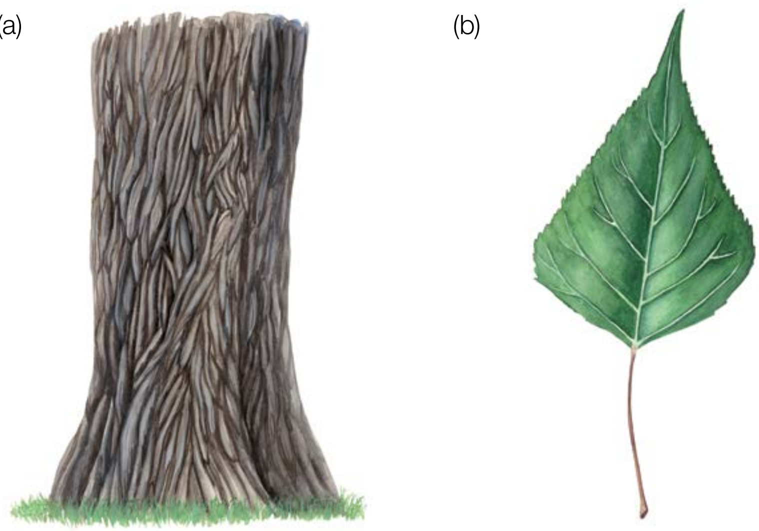

Figure 2: The bark on mature trees is dark greyish-brown or black with numerous deep intercrossing fissures (a). Characteristic diamond (rhomboid) to triangular shaped European black poplar leaf (b).

European black poplar is a dioecious species. Unisexual male or female flowers (Figure 3) develop from specialised buds containing preformed inflorescences [11]. Flowers are clustered in pendulous catkins on separate trees, which allows for strict outcrossing. Male catkins have reddish-purple anthers, while female catkins have yellow-green stigmas.

(a) (b)

Figure 3: Identification of tree's sex: male flowers (a), female flowers (b) schematically presented in different development phases.

3 Reproduction

Male trees are trees with only male flowers, and they produce pollen, female trees are trees with only female flowers, and they produce seeds. The flowers appear from specialised buds approximately 1-2 weeks prior to leaf initiation in the early spring (March-April) in lower elevations and latitudes, while at higher latitudes and elevations flowering is delayed until May [15]. The timing and duration of flowering and length of the seed maturation process are related to both the photoperiod and local temperatures and, therefore, will vary from one locality to the next with implications for the timing of seed release [16]. There may also be a genetic component resulting in early and late timing phenotypes. Pollen is dispersed by the wind. Once female flowers are fertilised, approximately 20-50 bare and round green-brown fruit capsules will ripen on each catkin in 4-6 weeks (Figure 4a), producing up to 250 small light-brown seeds per catkin [17]. Female catkins develop into fluffy cotton-like airborne seeds with long, white, silky hairs attached to the seed (Figure 4b), which fall in the early summer [17].

European black poplar produces seed almost every year. Seeds have a short (1-3 days) viability period and need specific water and soil conditions with continuously wet substrate for a 4-week period to allow germination [18].

European black poplar can reproduce generatively as described above or vegetatively (clonally). Natural clonal reproduction is possible by suckering from root sprouts, from stumps, fallen trees and broken branches at the

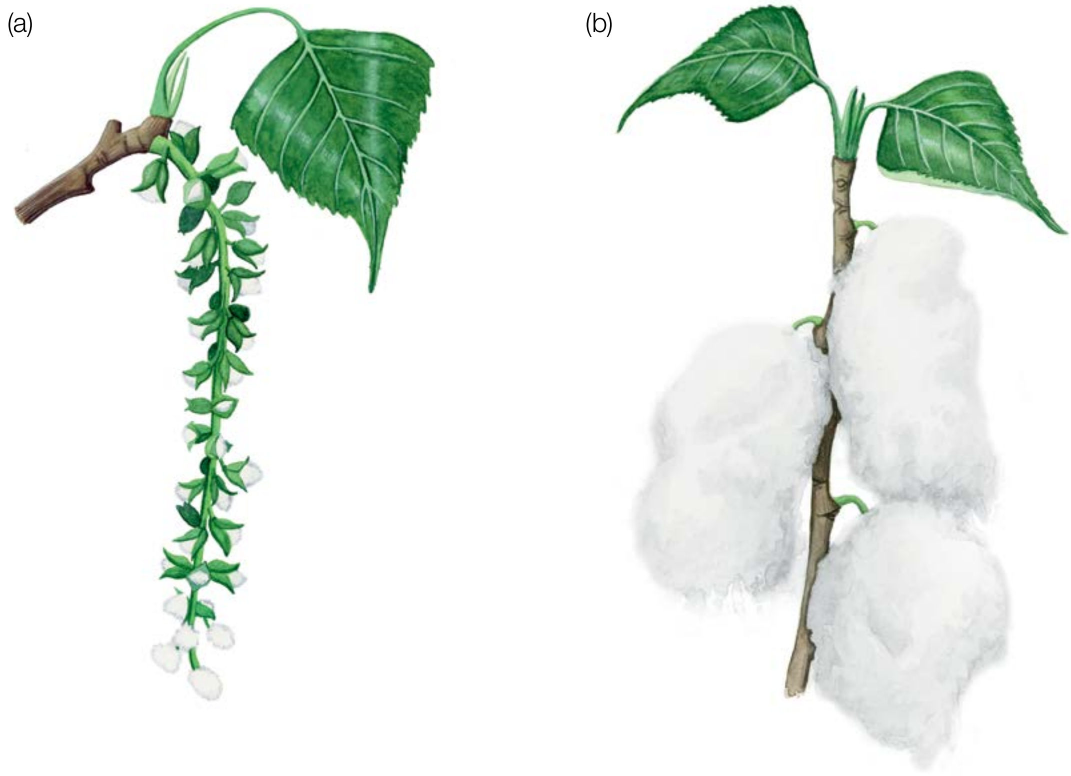

Figure 4: Female catkin with ripening seed capsules (a); mature seeds of *Populus nigra* have long, white silky hairs attached to them, giving a fluffy, cotton-like appearance (b).

juvenile stage [17]. Natural vegetative reproduction is possible even when seedling establishment is absent. and therefore can contribute to overall recruitment. *Populus nigra* often exhibits a polycorm of clonal plants [2].

Identification of regeneration sites

European black poplar naturally regenerates only on riverbanks on patches of ruderal, moist sandy and loamy soils exposed after seasonal river flooding [14], but not under trees of reproductive age in older floodplain forest stands. Seedling recruitment occurs along meandering rivers in arcuate bands of successive ages, while in braided river systems in association with specific microsites (e.g. in patches of sand which have accumulated behind clumps of vegetation, or woody debris, in silt-filled depressions on the floodplain) [17]. Successful natural regeneration is usually patchy and sporadic. Due to changes in site conditions the species' population size may fluctuate (expand or contract) over time [7].

4 Environment

European black poplar has a wide natural distribution range throughout Europe, except for the Nordic countries and from North Africa to Central Asia, including the Caucasus and the large part of the Middle East. Its range extends as far as Kazakhstan and China [11], and from sea level to 4000 m in elevation [19]. Throughout its natural range, cultivated forms or hybrids often replace the natural *Populus nigra* stands [20]. European black poplar is primarily preserved along the main rivers and their tributaries on alluvial sites. European black poplar naturally forms metapopulations rather than small, isolated populations (Figure 5) [6, 7]. In stands, it is present as many individual trees (solitaires) or smaller groups of over-mature trees. It grows together with white poplar (*Populus alba* L.), willows (*Salix* spp.), alder (*Alnus* spp.), maple (*Acer* spp.), elm (*Ulmus* spp.), and sometimes oak (*Quercus* spp.) [21]. The best growth is observed on deep medium texture soils with pH between 5.5 and 7.5 and high nutrient content. Because of its sporadic occurrence in mixed riparian stands it is generally not included in the regular forest inventories.

5 Threats

Despite its wide distribution range, European black poplar is a vulnerable and rare tree species currently close to extinction in several parts of its range due to human influences such as: i) over-exploitation of its natural sites; ii) alteration of riparian ecosystems by human activities; iii) cultivation of superior hybrids of *P.* × *canadensis* Moench (hybrids between *Populus deltoides* W. Bartram ex Marshall and *Populus nigra*), Eastern Cottonwood (*Populus deltoides*) and Balsam poplars (*Populus trichocarpa* Torr. & A. Gray ex. Hook, *Populus maximowiczii* Henry) within its natural range; and iv) gene introgression from the introduced female hybrid clones when their flowering is synchronised with the male European black poplar [22, 14, 11].

A frequently observed pest on European black poplar is *Chrysomela populi* L., while the most frequent diseases are poplar leaf rust (*Melampsora larici-populina* Kleb.) and *Marssonina* leafspot of poplar (*Drepanopeziza punctiformis* Gremmen, also known as *Marssonina brunnea* (Ellis & Everh.) Magnus. Dieback of old European black poplar trees is also frequently observed in its native sites due to changes in site conditions and drought (rapid decline of groundwater levels). Old trees are finally destroyed by the dothichiza bark necrosis of poplar caused by *Plagiostoma populinum* (Fuckel) L. C. Mejía (formerly *Cryptodiaporthe populea* (Saccardo) Butin, also known as *Dothichiza populea* Saccardo) as well as windbreaks, and consequently the natural succession of mixed riparian forest is towards hardwood formations.

6 Plot establishment and maintenance

European black poplar is a pioneer species, which is present in riparian mixed forests. It is characterised by a metapopulation structure across the wide floodplain system. FGM of European black poplar should be implemented on the scale of a metapopulation representing a whole network of inter-linked local subpopulations, among which exchange of pollen and seed is putatively present, and must not be applied to a single locally isolated site.

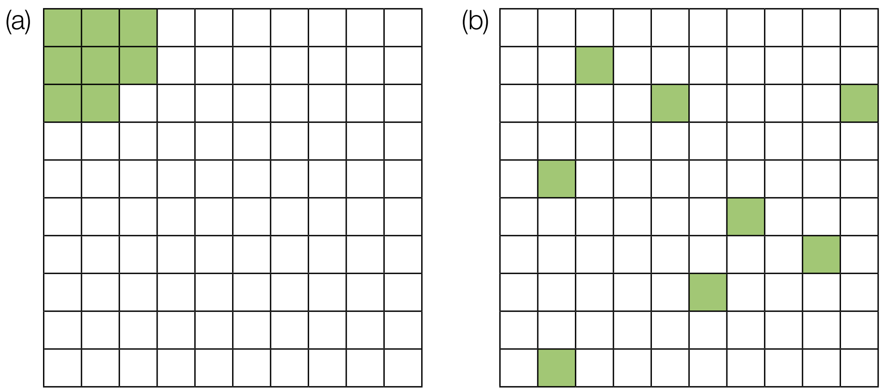

Figure 5: Schematic presentation of inter-linked black poplar local populations along a river system (a) vs. the local black poplar population in isolation (b).

To ensure representative sampling across the metapopulation it is important to design a genetic monitoring system with randomly selected monitoring plots of mature trees in local populations and their natural regeneration centres along the river system. An FGM plot for European black poplar consists of as many plots as there are local populations that form the metapopulation of interest. The number of trees in each plot should be proportional to the local population size, with the total sum of 50 mature (reproducing) genetically different pure *Populus nigra* trees with preferably equal representation of male and female individuals (sex ratio 1:1). The monitoring plot in each local population should include at least 20 trees distributed across a maximum distance of 5 km.

The trees are proposed to be pre-selected on site by assessment of morphological traits, detailed in the species description. Based on the results of long-term gene conservation projects in Hungary [23], for which a complex of stable morphological traits were used and the pre-selected trees were additionally tested by diagnostic DNA markers, the pre-selection could in most cases exclude hybrid and introgressed genotypes. However, diagnostic molecular genetic markers for characterisation of taxonomic status should be used in all cases to confirm the taxonomic identity and non-hybrid nature of the tested trees as pure *Populus nigra* individuals [7, 23]. Therefore, use of genetic tests by molecular diagnostic markers must be an essential element of genetic monitoring of *Populus nigra* on all monitoring levels. Additionally, the trees must also be tested for clonality by genotyping (only one individual of the same genotype can be included in the monitoring). If a tree is flowering it is regarded as a reproducing tree. In order to distinguish between the sexes, the plot installation in the field should ideally be carried out in the flowering season. During plot installation, trees should be labelled and georeferenced. At the same time height and DBH can be measured and samples for DNA extraction taken.

6.1 Plot establishment

6.1.1 Definition of the sampling frame

Before an FGM plot is installed in the field, a map of the European black poplar metapopulation should be prepared in GIS software. For this reason, the local population locations, where the species appears in sufficient density to set up a monitoring plot, should be surveyed in more detail in the field. It is recommended to record a walking track using a mobile phone app (e.g. Locus map) or a GPS device during this initial surveying, which greatly facilitates further planning.

The locations of local populations are plotted on the map in the form of polygons, which all together represent a sampling frame. Trees within each local population should be selected randomly. The approach that enables random selection is creating an appropriate number (proportional to the local population size) of random GPS coordinates in GIS software with a minimum distance of 35 m between them. The rationale behind using a longer distance between random points is to provide a safety margin for the reduced accuracy of GPS devices in forests and the distance of the nearest tree from the random GPS point. Random points' coordinates are saved into a GPS device, which is to be used in the field. If the instructions described are not feasible due to the complexity of the river channels in alluvial forests, a simplified "seek and find approach" within all local populations may be used: preferably with the help of a local forester, the area, where local populations occur, is combed in a systematic pattern using a GPS device or mobile phone app with track recording, which ensures that the same area is not inspected repeatedly, or any part of the area is not overlooked. Coordinates of all reproducing trees are logged, and their sex determined. An appropriate number of trees is selected randomly from the pool of suitable ones for each local population.

All adult trees must be genotyped to exclude hybrids and clones on all monitoring levels.

6.1.2 Plot installation in the field

Since the coordinates of approximate tree locations are known, the procedure for plot installation in selected local population is:

- finding saved GPS coordinates in the forest stands,
- selecting and marking the closest reproducing tree to the saved GPS coordinate.

6.1.3 Labelling of trees

Each selected tree must be marked with a corresponding number and a band painted around the trunk to aid the visibility of the trees from all directions.

6.1.4 Sampling for genetic analyses

Samples for DNA extraction for all selected trees must be collected for assessment of hybridisation and presence of clones. Hybrids and clones must be excluded and replaced by non-hybrid individuals with unique genotypes (not clones). Consequently, it may be necessary to select and sample a larger number of trees to find the 50 European black poplar trees that are not hybridogenous or clones.

6.2 Natural regeneration centres

Sampling design of natural regeneration (NR) follows the metapopulation concept of multiple regeneration centres (subplots) to capture the whole genetic diversity of European black poplar and assess the risk of gene introgression and hybridisation from exotic poplar species, and Lombardy poplar sources in the given area. For NR sites, we must take into account flood disturbances and therefore the constantly changing shapes of the microsites' locations, environmental conditions or potentially even their disappearance.

The microsites of possible NR should be frequently monitored (at least once per week) at the end of the fructification phase in early summer (mainly from April to June), and where newly germinated NR centres are discovered the European black poplar offspring with the cotyledons or initial leaves should be sampled immediately. NR centres sampled should be mapped by recording their GPS coordinates. Ideally, 20 NR subplots with a size of 1 m^2 each should be located across the FGM area, with an additional 0.5 km in both directions of the river system.

European black poplar should be sampled in NR subplots due to very diverse covering of offspring on each site. Ideally, 5 plants randomly selected from each subplot of 1 m^2 should be collected for a total of 100 samples. If fewer than 20 NR locations are discovered, a proportionately higher number of samples per each NR subplot should be sampled. All samples are tested for hybridisation, and among them 50 pure European black poplar are randomly selected for further FGM analyses; if it is not possible to get 50 pure species plants from those 100, sampling and testing of an additional batch of 100 samples must be performed until the minimum required number of 50 genotypes of pure *Populus nigra* individuals from NR centres needed for the FGM analysis is reached.

6.3 Plot maintenance

6.3.1 General maintenance

Tree markings markings must be checked periodically (every 2 years) and renewed if needed.

6.3.2 Replacement of trees

If a monitored tree dies or is cut due to management, it must be replaced. The nearest suitable tree to the dead one should be chosen considering that the distance requirement of 30 m to the nearest monitored tree is fulfilled. Otherwise a tree from the periphery of the FGM plot is to be selected.

If the crown is damaged due to, for example, windbreak, ice or snow break, but continues to fructify, the tree is kept for the monitoring. If the damage is too severe and fructification is not expected anymore, the monitored tree must be replaced. The cause of damage needs to be recorded, as the damage can affect the values recorded for field verifiers and background information.

7 Recording of verifiers and background information

Molecular genetic identification of European black poplar trees should be performed with the use of species diagnostic genetic markers. Clonality detection in both the adult tree metapopulation and juvenile regeneration centres should be evaluated by genetic markers as a part of molecular genetic analyses. A set of verified reference samples of both (or even more) hybridising species is needed to discriminate between pure species and interspecific hybrids.

- In general, the following should be kept in mind that:
- The European black poplar population has a metapopulation structure.
- FGM plots are “local population plots” in the metapopulation.
- FGM plot chosen in a metapopulation along the river system form a FGM plot with 50 mature trees of European black poplar in total.
- All mature FGM European black poplar trees are considered for observations and measurements.
- Molecular genetic analyses are required to be performed on all monitoring levels in order to include “pure species” individuals in the monitoring. Therefore, FGM for this species becomes significantly more expensive to start with in comparison with that for non-hybridising tree species.

Verifiers and background information are periodically recorded on the monitoring plot. Verifiers are used to monitor the population’s genetic properties and its adaptation to environmental changes and/or management, while background information needs to be recorded to assist interpretation of the verifiers.

Higher levels of verifiers (standard, advanced) must also include recording on all the preceding levels (basic, standard). This is not necessary for the recording of background information.

Table 1: List of verifiers and background information with short description and observation frequency to be recorded during field work at the FGM plots

	Name	Basic level	Standard level	Advanced level
Verifiers	Mortality / survival	Adult trees: Counting of remaining marked mature trees every 10 years and after every extreme weather event/ disturbance	Same as basic level	Same as basic level
		Natural regeneration: mortality / survival is not estimated for this species	/	/
	Flowering	FGM plot level expert opinion, every year.	Individual tree level observation, during two major flowering events per decade, ideally equally spaced. *	Same as standard level, but flowering stage also recorded. *
	Fructification	Individual tree level observation twice per decade, the same year as major flowering was observed. (regardless of the fructification intensity).*	Individual tree level observation, the same year as the assessment of the flowering at the standard level (regardless of the fructification intensity). *	Counting of fruit (cotton-like catkins with mature seed capsules), during the same years as the assessment of flowering at the advanced level, regardless of the fructification intensity. * Seeds are collected for laboratory analyses for every assessed fructification event at the advanced level
	Natural regeneration abundance	Expert opinion on the FGM plot level.**	Counting of seedlings on up to 20 NR centres of only the newly germinated NR after every assessed major fructification event. Samples for genetic analyses are also collected at the same time. **	The same as standard level. **
Background info	DBH class distribution	/	Measurement every 10 years	Same as standard level
	Height class distribution	/	Measurement every 10 years	Same as standard level
	Budburst	/	Individual tree level observation according, every 5 years	Individual tree level observation, every year
	Senescence	/	Individual tree level observation, every 5 years	Individual tree level observation, every year
	Flowering synchronisation	/	/	Individual tree level observation, during each assessed major flowering event

* Ideally at least one major fructification event should be assessed per decade. However, a major flowering event does not necessarily lead to a major fructification event. If no major fructification event follows the assessed flowering event, assessment of both flowering and fructification needs to be repeated during the next major flowering event, regardless of the time passed between successive major flowering events. Basic level observations are used to identify major flowering and fructification events.

** If no new NR centres are present after an assessed major flowering and fructification event (in an event such as floodwater washing the seedlings away), then the assessment of all three verifiers (flowering, fructification and NR abundance) must be repeated the next major flowering event, regardless of the time passed between successive major flowering events. Basic level observations are used to identify major flowering and fructification events.

7.1 Protocols for recording of verifiers

7.1.1 Mortality / survival

Mortality describes the mortality of adult trees. Its counterpart survival stands for trees that are still alive since the previous assessment. Survival is calculated as 1 – Mortality.

7.1.1.1 Adult trees: Basic, standard, and advanced levels

The verifier for mortality of adult trees. It is estimated by counting the marked trees remaining alive every 10 years and after every extreme weather event/disturbance. Mortality is the difference between the initial number of marked trees and the trees remaining alive of the original 50 trees.

7.1.2 Flowering

This verifier describes the flowering intensity and proportion of trees thus affected. It can be recorded in April in Central Europe. Flowering is earlier when preceded by a warm winter.

7.1.2.1 Basic level

This verifier is recorded every year at the stand level. Recording is carried out when flowering is in full progress. The estimate of average condition is provided after a walk throughout the monitoring plot. Two scores are given, one for flowering intensity and one for the proportion of flowering trees in the stand.

Code	Flowering intensity at the stand level	Average proportion of crown flowering (%)
1	No flowering: No or only occasional flowers appearing on trees	0 – 10
2	Weak flowering: Some flowers appearing on trees.	> 10 – 30
3	Moderate flowering: Moderate number of flowers appearing on trees.	> 30 – 60
4	Strong flowering: Abundant number of flowers on trees.	> 60 – 90
5	Massive: Huge number of flowers on trees.	> 90

Code	Proportion of trees in the stand with the given flowering intensity stage (%)
1	0 – 10
2	> 10 – 30
3	> 30 – 60
4	> 60 – 90
5	> 90

7.1.2.2 Standard level

This verifier is recorded during two major flowering events per decade, ideally equally spaced in time from one another. It is recorded at an individual tree level on all 50 monitored trees. A major flowering event is when at the basic level flowering intensity is strong or massive (code 4 or 5) and the proportion of trees with the given flowering intensity is above 60% (code 4 or 5). Recording is carried out when flowering is in full progress. One score is provided for each tree.

Code	Description of flowering intensity	Proportion of the crown flowering (%)
1	No flowering: No or only occasional flowering appearing on a tree.	0 – 10
2	Weak flowering: Some flowers appearing on a tree.	> 10 – 30
3	Moderate flowering: Moderate number of flowers on a tree.	> 30 – 60
4	Strong flowering: Abundant number of flowers on a tree.	> 60 – 90
5	Massive: Huge number of flowers on a tree.	> 90

7.1.2.3 Advanced level

This verifier is recorded during two major flowering events per decade, ideally equally spaced in time from one another. It is recorded at an individual tree level on all 50 monitored trees. A major flowering event is when at the basic level flowering intensity is strong or massive (code 4 or 5) and the proportion of trees with the given flowering intensity is above 60% (code 4 or 5).

Two scores are provided for each tree: flowering stage to describe the stage of flower bud development for male and female trees with flowering intensity and the proportion of the crown flowering. On average, two visits to the plot are needed; the first one early enough to observe the early stages of flowering, and the second when flowering is in full progress. Background information on flowering synchronisation can be estimated from the scores for male and female flowering recorded by this verifier. For a graphical representation of male and female trees flowering stages, see Figures 6 and 7.

Code	Female flowering stage
1	Female flowering buds not active (brown coloured buds)
2	Female flowering buds increase in size and start to break (light-green coloured buds)
3	Flower elongation (short light-green coloured flowers)
4	Flowers open (greenish coloured catkins)
5	Flowers open (fully developed yellow-green coloured flowers in catkins)

Code	Male flowering stage
1	Male flowering buds not active (brown coloured buds)
2	Male flowering buds increase in size and start to break (light-green coloured buds with first reddish-purple flowers visible)
3	Flower elongation (short reddish-purple flowers)
4	Flowers open (fully developed reddish-purple flowers catkins with pollen)
5	Flowers dry out and fall off

Code	Flowering intensity for each tree, valid for both sexes	Proportion of the crown flowering (%)
1	No flowering: No or only occasional flowering appearing on a tree.	0-10
2	Weak flowering: Some flowers appearing on a tree.	>10-30
3	Moderate flowering: Moderate number of flowers on a tree.	>30-60
4	Strong flowering: Abundant number of flowers on a tree.	>60-90
5	Massive: Huge number of flowers on a tree.	>90

Figure 6: Picture guide for male reddish-purple coloured catkin flowering development stages for the advanced level verifier Flowering.

Figure 7: Picture guide for female yellow-green coloured catkin flowering development stages for the advanced level verifier Flowering

7.1.3 Fructification

This verifier describes the presence of fructification and its abundance. Data for this verifier should be collected during fructification, mainly from late April to June in Central Europe.

7.1.3.1 Basic and standard levels

This verifier is recorded twice per decade during the years of major flowering. Ideally, assessments of fructification should be equally spaced in time from one another. It is recorded at an individual tree level on all monitored female trees (ideally 25). Recording is carried out before seeds start falling. One score is provided for each tree.

Ideally, one major fructification event should be captured following observations of major flowering events per decade. However, a major flowering event does not necessarily lead to a major fructification event. If no major fructification event follows the assessed flowering event, then the assessment of both flowering and fructification needs to be repeated during the next major flowering event, regardless of the time passed between successive major flowering events. A major fructification event is when fructification intensity is strong or massive (code 4 or 5) for at least 60% of the monitored female trees.

Code	Fructification intensity	Proportion of the crown fructifying (%)
1	No fructification: No or only occasional fruit appearing on a tree.	0 – 10
2	Weak fructification: Some fruit appearing on a tree.	> 10 – 30
3	Moderate fructification: Moderate amount of fruit appearing on a tree.	> 30 – 60
4	Strong fructification: Abundant amount of fruit appearing on a tree.	> 60 – 90
5	Massive: Huge amount of fruit appearing on a tree.	> 90

7.1.3.2 Advanced level

This verifier is recorded during the same years as the assessment of the flowering at standard and advanced levels (regardless of the fructification intensity). It is recorded at an individual tree level on all monitored female trees (ideally 25). Recording is carried out before seeds start falling. One score is provided for each tree. Simultaneously, seed is collected from 20 female trees for seed and genetic analysis for the advanced level verifiers and background information.

Ideally, one major fructification event should be captured following observations of major flowering events per decade. However, a major flowering event does not necessarily lead to a major fructification event. If no major fructification event follows the assessed flowering event, assessment of both flowering and fructification needs to be repeated during the next major flowering event, regardless of the time passed between successive major flowering events. Basic level observations are used to identify major fructification events. A major fructification event is when at the basic or standard level fructification intensity is strong or massive (code 4 or 5) for at least 60% of the monitored female trees.

The verifier is recorded by counting fruits (cotton-like catkins with mature seed capsules) using binoculars. The average of three rounds of counting is reported. Each round of counting consists of the number of fruits that the observer counts in 30 seconds. For all trees, the same part of the crown should be investigated. Once the observation part of the crown part is selected, the same one should be selected for every subsequent monitoring of this verifier. The upper third of the crown is preferred to the bottom and middle part for counting.

Two values are recorded; the number of fruits and the part of the crown monitored.

Number of fruits counted in 30 seconds (average of 3 rounds)
X

Code	Part of the crown monitored
1	Bottom
2	Middle
3	Top

7.1.4 Natural regeneration presence and abundance

This verifier describes the presence and abundance of natural regeneration at the monitoring plot.

7.1.4.1 Basic level

This verifier is recorded at the FGM plot level every year, in late spring to early summer. Expert opinion is used for estimation considering the situation on the FGM monitoring area as a whole.

Code	Description: new regeneration (newly germinated seedlings)
1a	There is no or very little new natural regeneration on the monitoring plot
2a	New regeneration is present in sufficient quantity on the monitoring plot

Code	Description: established natural regeneration (saplings)
1b	There is no or very little established natural regeneration on the monitoring plot
2b	Established regeneration is present in sufficient quantity on the monitoring plot

Figure 8: Newly germinated seedling of European black poplar with characteristic cotyledons or initial developed leaves.

7.1.4.2 Standard level and Advanced level

This verifier is recorded by counting newly germinated seedlings (Figure 8) after every assessed major fructification event on up to 20 NR centres. NR subplots are not established for European black poplar due to high expected loss of NR because of regular river flooding. Consequently, counting is performed only once, immediately after germination, and survival/mortality of NR is not assessed for this species. At the same time, NR regeneration samples are collected for genetic analyses.

Counting of seedlings:

All European black poplar seedlings present at each of the 20 NR centres must be counted. Any older European black poplar saplings that are present on the NR subplot must not be included.

If no new NR centres are present after an assessed major flowering and fructification event (in an event such as floodwater washing the seedlings away), then the assessment of all three verifiers (flowering, fructification and NR abundance) must be repeated the next major flowering event, regardless of the time passed between successive major flowering events. Basic level observations are used to identify major flowering and fructification events.

If no new NR centres are formed in 5 consecutive monitoring years (after two major fructification events in a decade) then NR should be estimated and samples collected for genetic analyses once per decade in already successfully established NR sites. In such cases, the approximate age of NR must be assessed and recorded.

Number of seedlings counted on a subplot
X

7.2 Protocols for recording of background information

7.2.1 DBH class distribution

7.2.1.1 Standard and advanced levels

DBH is recorded on an individual tree level on all 50 monitored trees every 10 years. DBH is the trunk diameter at 1.30 m, i.e. approximately at an adult's breast height. If a tree has more than one trunk, please measure all of them and record the average (but try to avoid trees with many small trunks). Note that the tree is multi-trunk in the notes and include the number of trunks measured. If the tree is leaning, measure DBH perpendicular to the tree trunk. DBH can be measured in two ways:

1) using a calliper, in which case you need to measure two perpendicular diameters and take the average
2) measure the circumference of the tree and compute the diameter from that value (i.e. divide by π, ~3.14 or use a pi-meter)

The DBH is recorded in cm. The same method must be applied for every subsequent measurement.

7.2.2 Height class distribution

7.2.2.1 Standard and advanced levels

Height is recorded on an individual tree level on all 50 monitored trees every 10 years. Height is measured from the ground to the tallest part of the crown, ideally using a clinometer or hypsometer (e.g. vertex). Height is recorded in metres to one decimal place. If the crown is damaged, this must be recorded as well as the stipulated reason in the notes.

7.2.3 Budburst

Budburst describes the process of budbursting (flushing). Recording of this parameter is only carried out at the standard and advanced levels. In European black poplar, budbursting starts later than flowering. Data for this verifier should be collected in March – May in Central Europe. Budbursting is earlier when preceded by a warm winter. Recording of this parameter is only carried out at the standard and advanced levels.

7.2.3.1 Standard level

At standard level, budburst is recorded on an individual tree level on all 50 monitored trees every 5 years. For each tree, two estimates are given: budbursting stage and proportion of the crown budbursting. For a graphical representation of budbursting stages, see Figure 9.

Code	Stage	Stage of budbursting
1	Dormant bud	buds from completely enveloped by the scale to the first sign of swelling
2	Swelling	buds swelling with scale slightly diverging
3	Bursting	buds sprouting
4	Separation of leaves	buds are completely opened with leaves still clustered
5	Leaves elongate	leaves diverging with their blade
6	Vertical growth	leaves completely unfolded and fully developed

Code	Proportion of the crown with a given stage of budbursting (%)
1	> 0 – 33%
2	> 33 – 66%
3	> 66 – 99%
4	100%

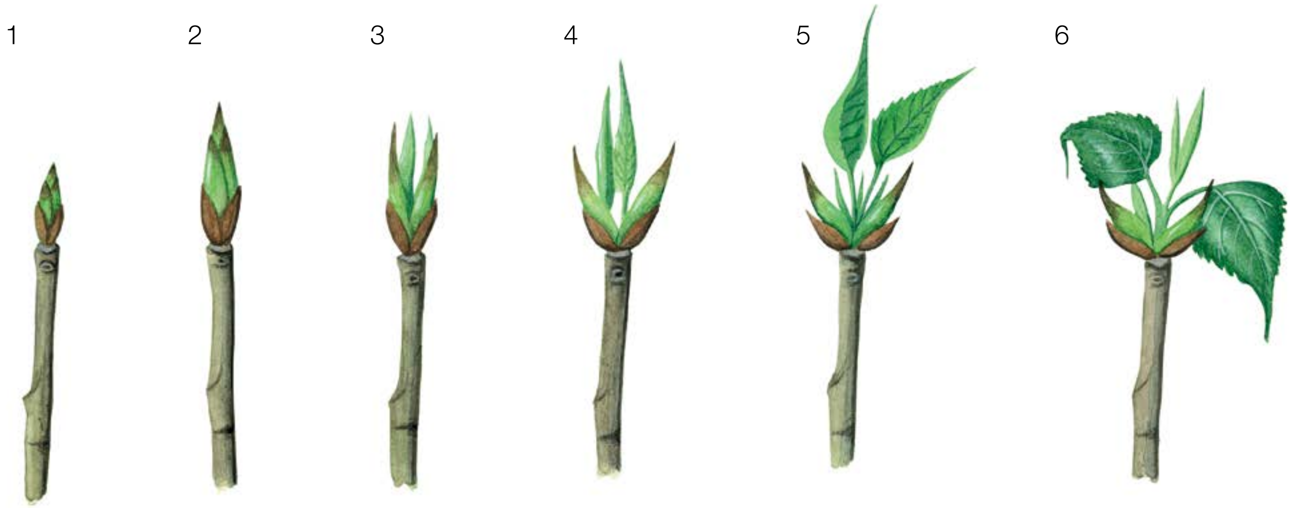

Figure 9: Picture guide for description of budburst (flushing) for the standard, and advanced levels background information Budburst

7.2.3.3 Advanced level

At advanced level, budburst is recorded on an individual tree level on all 50 monitored trees every year. We are looking for the initiation of budbursting (stage 3) and the end of budbursting (stage 6). The observations stop when all trees have reached stage 6. Usually 2 visits will be needed. For the values (stage of budbursting and the proportion of crown affected) see 7.2.3.1 Standard level.

7.2.4 Senescence

Senescence describes the process of senescence. Recording of this parameter is only carried out at the standard and advanced levels.

7.2.4.1 Standard level

At standard level, senescence is recorded on an individual tree level on all 50 monitored trees every 5 years. For each tree, two estimates are given: stage of senescence and proportion of the crown senescing. For graphical representation of stages of senescence see Figure 10.

Code	Stage of senescence
1	Leaves are green
2	Leaves are green changing to yellow (greenish yellow)
3	Leaves are yellow changing to brown (brownish)
4	Leaves are brown / shed

Code	Proportion of the crown with a given score for stage of senescence (%)
1	> 0 – 33%
2	> 33 – 66%
3	> 66 – 99%
4	100%

7.2.4.2 Advanced level

Senescence is recorded on an individual tree level on all 50 monitored trees every year. We are looking for stage 3, when leaves are yellow and do not photosynthesise anymore. Observations stop when all trees have reached stage 3. Usually 2 visits to the plot will be needed. For the values (stage of senescence and the proportion of crown affected) see 7.2.4.1 Standard level.

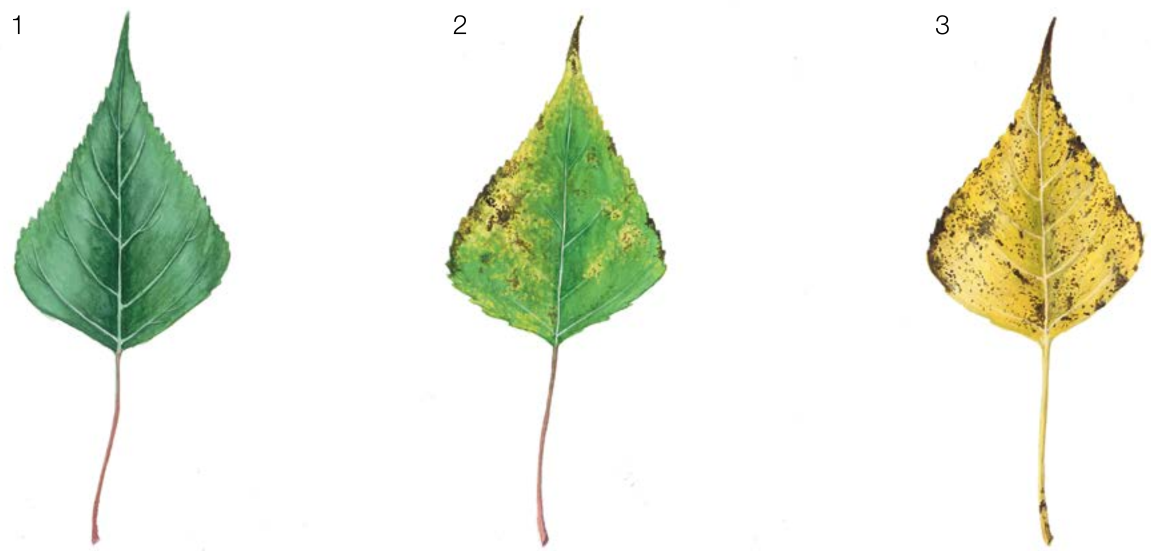

Figure 10: Picture guide for description of senescence (phase 4 is not shown) for the standard and advanced level background information Senescence

7.2.5 Flowering synchronisation

7.2.5.1 Advanced level

Flowering synchronisation is monitored only at the advanced level, and is based on the data collected for the verifier Flowering (see 7.1.2.3). It is used to determine whether male and female flowering times occur simultaneously within the monitored stand.

For plot establishment use form 'FGM Plot description'

For verifiers recording use 'Form for recording field level verifiers within FGM'

For background information recording use 'Form for recording field level background information within FGM'

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