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European black pine (*Pinus nigra* J. F. Arnold)

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9.2.4 European black pine (*Pinus nigra* J. F. Arnold)

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1 Executive summary

European black pine (*Pinus nigra* J. F. Arnold) is a wind-pollinated, monoecious, mainly outcrossing, high elevation, circum-Mediterranean conifer, that also grows in Austria, Crimea, and the Black Sea. Due to the species' extensive distribution in a broad spectrum of environments, that led to its morphological and genetic differentiation, five interfertile sub-species can be recognised across its natural distribution. Black pine is a valuable key-stone species of high economic and ecological importance, producing wood of high quality and natural durability. It is characterised by its tolerance to abiotic stresses, such as poor and salty soils, frosts, ice weight, strong winds, and drought. The species regenerates naturally in forest ecosystems, but has no mechanisms of regeneration after fire, a fact that renders it vulnerable to the extensive wildfires usually occurring across the Mediterranean basin. Given the high economic and ecological significance of the species, its extensive natural distribution to a variety of habitats and the existence of isolated and marginal populations that could be at risk in the face of climate change, it can be considered as a good candidate species for genetic monitoring.

The present guidelines provide a short description of the European black pine; its distribution, ecology, reproduction and threats posing risks for the species, together with guidance on the establishment of a monitoring plot and the recording of all field verifiers needed to fulfil the genetic monitoring goals.

2 Species description

European black pine is a circum-Mediterranean conifer, growing also in Austria, Crimea, and the Black Sea. The following [1] five subspecies can be recognised based mainly on morphological/anatomical traits: a) *P. nigra* J. F. Arnold subsp. *nigra* , distributed in southeastern Austria, northern Italy, the Balkan Peninsula, Bulgaria, Romania, Turkey-in Europe; b) *P. nigra* subsp. *dalmatica* (Vis.) Franco, distributed in Croatia; c) *P. nigra* subsp. *laricio* (Poir.) Palib. ex Maire, distributed in France (Corsica) and Italy (Apennines, Sicily); d) *P. nigra* subsp. *pallasiana* (Lamb.) Holmboe distributed to Greece, Cyprus, southwest Bulgaria, southeast North Macedonia, south Albania, and from Crimea along the Black Sea coast to Turkey; and e) *P. nigra* subsp. *salzmannii* (Dunal) Franco, distributed in southwest Europe, France (Hérault, Pyrenees), Spain, Algeria and Morocco. The species grows in association with *Pinus sylvestris* L., *Pinus mugo* Turrra, *Pinus halepensis* Mill., *Pinus pinea* L. and *Pinus heldreichii* Christ [2]. In most of the cases the species forms pure stands, while it can be found in mixed stands together with other pines and especially *Pinus sylvestris* [12].

(a)

(b)

Figure 1: *Pinus nigra* habitus (a) and needles (b).

Natural interspecific hybridisation among *Pinus nigra* and other pine species has been reported, as for example with *P. sylvestris*, *P. heldreichii, P. densiflora* Siebold & Zucc., *P. resinosa* Aiton, *P. tabulaeformis* Carrière, *P. taiwanensis* Hayata, *P. mugo, P. thunbergii* Parl. [3,4,5,6,7], when the species naturally co-exist with black pine or when artificially introduced. Intraspecific hybridisation among subspecies is possible, as the reproductive barriers to gene exchange among them are weak, leading to transitional forms that result from the extensive gene flow due to the long-distance pollen dispersal [8].

The species is a medium-sized two needle pine (Figure 1), reaching at the maturation age (80 years of age) a height of 30 - 50m, being characterised by a straight stem form. The bark colour ranges from light grey to dark grey-brown and is widely split by flaking fissures into scaly plates in old trees [9]. The bark becomes increasingly creviced with age [10]. The crown has pyramidal form in the young age but rounds with age forming a spreading flat top or dome. The needles are stiff, 8 to 16 cm long and 1-2 mm wide, straight or curved and finely serrated, while the needle sheath is 10-12 mm long [11,12].

3 Reproduction

European black pine is a monoecious anemophilous conifer with winged seeds, dispersed by the wind. Reproductive maturity is reached at the 15-20 years of age. The male strobili and the female strobili (conelets) (Figure 2a) appear every year during May. The female strobili (conelets) are red to purple and the male strobili when immature are green turning gradually to yellow when they reach maturity and shed their pollen. The pollen dispersal and female conelet receptivity occur from May to early June, while the duration of conelet receptivity usually lasts for three days [8]. Fertilisation occurs 13 months after pollination. The mature cones (Figure 2b) are sessile and horizontally spreading, 4-8 cm long and 2-4 cm wide, with a colour ranging from brown to yellow brown or even light yellow. The cones ripen from September to November of the second year and open the third year after pollination [12]. Usually each fertile cone scale produces two winged seeds (Figure 2c) and the cones usually bear 30-40 seeds out of which almost half germinate. The seed dispersal occurs from October till November of the second growing season. The seed colour may range from grey to light yellow and seed length from 5-7mm, while the wing length from 19-26mm. Mast seeding occurs every two to five years [13].

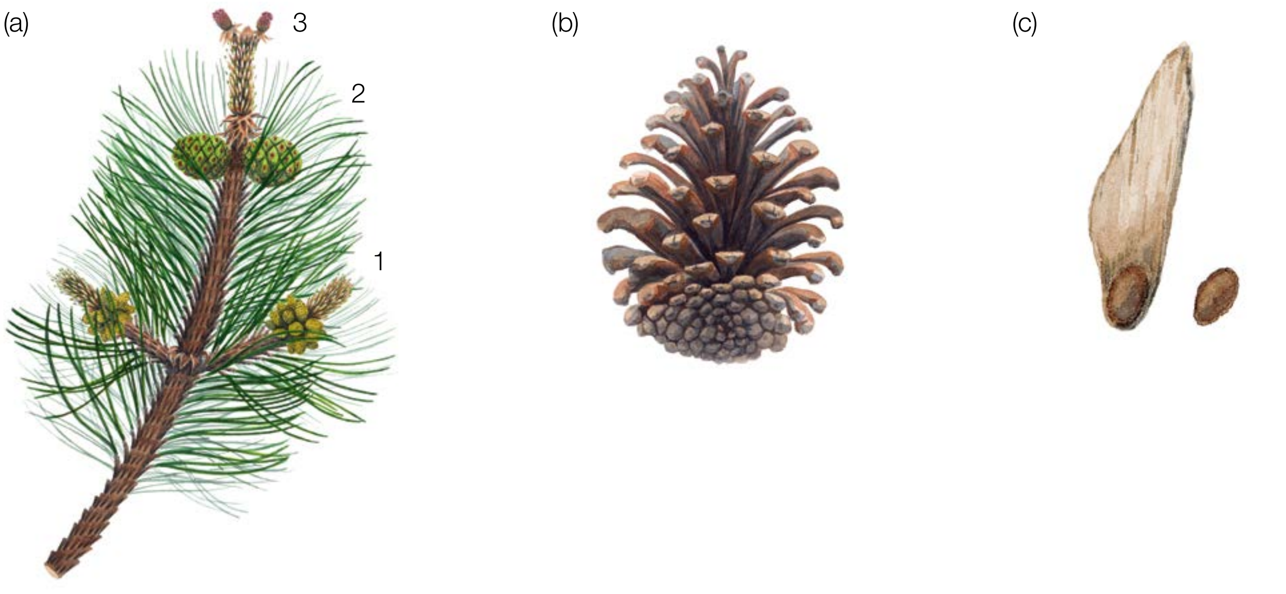

Figure 2: *Pinus nigra* branch with male strobili (a-1), female immature first year cones (a-2) and current year conelets (a-3), mature open cone (b) and seed with and without wing (c).

4 Environment

Pinus nigra is characterised by an extensive natural distribution (i.e. circum-Mediterranean distribution with occurrences in Austria, Crimea and Black Sea) that includes a broad array of environments. It grows at altitudes ranging between 350m to 2200m (Taurus Mts), but its optimum altitude is between 800m to 1500m. The species can grow in dry environments with poor soils and on a variety of substrates, ranging from limestone, to dolomites, acidic or volcanic soils [8]. Most of the species distribution falls within the Mediterranean-type climate, while the bioclimatic conditions may range from humid, to sub-humid and semi-arid. In parts of its native range it grows in cool to cold temperate climates, while the northern populations are frost-hardy, withstanding temperatures of -30°C, in contrast to the southern ones that may tolerate up to -7°C [2]. Photosynthesis has been recorded even at the -5°C, and respiration could still be detected at -19°C [2,14]. The species can also withstand well the weight of ice and it is generally considered as a hardy one. The species is photophilous, shade intolerant, and can tolerate well winds, drought and salty soils.

5 Threats

The species may face risks, especially when growing in isolated populations, due to several factors that may cause extinction, such as wildfires, insects and diseases, illegal cutting and the overarching threat of climate change. Insects like *Rhyacionia buoliana* Denis & Schiffermüller, *Thaumetopoea pityocampa* Denis & Schiffermüller, *Acantholyda hieroglyphica* Christ, *Diprion pini* L., *Pissodes validirostis* L., *Marchalina hellenica (Monophlebus hellenicus)* Gen., and *Ips pini* Say, *Bursaphelenchus xylophilus* may infest the species [8,15]. Fungi like *Mycosphaerella pini* Rostr. (*Dothistroma pini* Hulbary), *Lophodermella spp.*, *Sphaeropsis sapinea* (Fr.) Dyko & B. Sutton *(Diplodia pinea* (Desm.) J. Kickx f.) may also infect the black pine needles [16,17,18].

Additionally, mixing of gene pools across the whole of Europe, due to the extensive plantations established in the last two centuries with genetic material of unknown origin that can be maladapted to local conditions, constitutes a threat to the gene pools of autochthonous populations [8], as well as to their adaptive and evolutionary potential.

6 Plot establishment and maintenance

European black pine is a stand-forming tree species that in most of the cases forms pure stands, but it can also grow in mixture with *P. sylvestris* and other coniferous or broadleaved tree species [2]. Therefore, the regular Forest Genetic Monitoring (FGM) scheme followed for stand-forming tree species can be followed for black pine too.

An FGM plot should consist of 50 reproductively mature (i.e. flowering) trees, selected to fulfil the requirement of the 30m distance among any two of them. The trees reach sexual maturity at the age of 15–20 years in their natural habitat [8]. The social class and DBH (≥ 15cm) could be used as proxy variables to locate potentially reproducing trees, in case the establishment of the plot does not take place during the flowering period, relying on the expertise of the local foresters. Furthermore, the presence of sufficiently dense natural regeneration (NR) must be considered, prior to designating an FGM area, in case NR subplots will need to be established to study the mating system patterns, gene flow and level of potential genetic variation changes among different generations. The selected reproductively mature trees within the plot need to be labelled and their coordinates should be recorded. Additionally, up to 20 NR subplots have to be selected and marked for NR abundance assessment and sampling.

DBH measurement and sampling for DNA extraction can also be performed during the time of plot establishment, as well as the assessment of flowering in case the plot is being established within the flowering period.

Equipment needed:

- a device for distance measurement (a pair of range-finding binoculars is recommended)
- a compass
- paint and a paintbrush or paint sprayer for tree labelling
- a mask, safety glasses and gloves for spraying/labelling the trees
- a tree calliper for DBH measurements
- a GPS device that is precise enough and allows saving trees' coordinates
- a photo-camera to obtain pictures, in case the establishment of the plot is taking place during the flowering period.

Genetic monitoring plots in cases of isolated, marginal or threatened populations of this species can be larger than the regular ones. In those cases, the size and shape of the FGM plot should be flexible, but for practical reasons it should preferably not exceed 10 ha.

6.1 Plot establishment

6.1.1 Selection of the centre of the plot

The general procedure for random plot site selection consists of the following steps (Figure 3):

- Random selection of a point (green dot) on a map along the forest road or path, which runs along the stand,
- Drawing a perpendicular line from the randomly selected point on a road,
- Random selection of one point per line (red dot) – this point represents the centre of the FGM plot.

The minimum distance between the selected central point and stand border is approximately 150 m. If the selected central point doesn't meet this demand, a new point must be selected following the protocol described above.

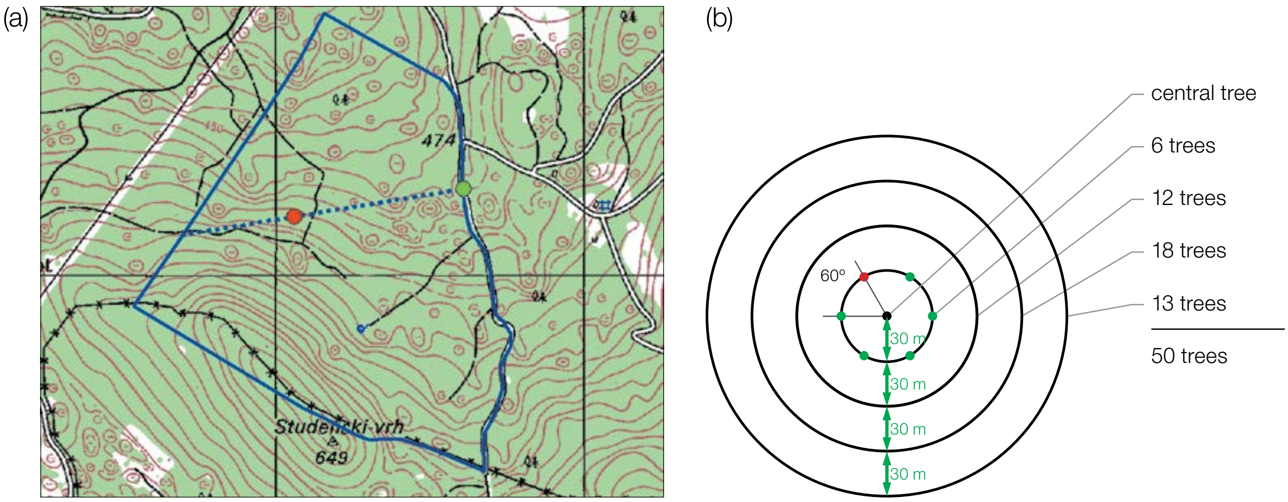

Figure 3: Random selection of the centre of the forest genetic monitoring plot (a); selection of trees in concentric circles around previously selected central tree with an increasing radius of 30 m (b).

Instead of the procedure described above, tools for creating random points in GIS software can also be used.

The selected point's coordinate should be saved in a GPS device that will be used in the field.

6.1.2 Plot installation in the field

In the field, the closest reproducing tree to the saved GPS coordinate becomes the centre of the monitoring plot and is marked with number 1.

Other trees are selected in concentric circles around the central tree with an increasing radius of 30 m (Figure 3b). The first tree in each circle should be selected randomly, which can be done in different ways: by using a random azimuth (Table 1) observed from the central tree, by following the direction of the second hand on an analogue watch or any other approach that allows for objective selection. The remaining trees in each circle are selected by an appropriately enlarged azimuth to assure a minimum distance of 30 m between any two trees:

- +60° for the first circle
- +30° for the second circle
- +20° for the third circle
- +15° for the fourth circle

If it is not possible to find 6, 12 and 18 trees in the inner 3 circles (Figure 3b), additional trees are selected in the outermost circle.

Table 1: Randomly generated azimuths that can be used for selection of the first tree in each circle.

108	15	186	35	178	29	305	351	44	150
232	23	160	141	112	292	216	83	245	214
63	65	345	234	95	78	279	323	40	236
201	313	275	144	182	68	268	289	185	92
356	177	93	1	145	198	287	251	224	142

In the case that the central tree is not visible due to existing obstacles (i.e. other trees covering the central tree) or topography, then the selection of trees can be based mainly on its minimum distance from the other selected trees (≥ 30m), while the approximate location of the central tree could only be assumed if considering the position of the selected trees from the previous circles or by plotting the coordinates on open Earth plotting platforms (i.e. google maps/earth).

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. Mark the central tree (number 1) with two or more bands to differentiate it from other trees (Figure 4a). It is recommended to paint the number on the side of the tree that is pointing away from the central tree, as this helps in locating the central tree, particularly from the outer rings of the plot (Figure 4b).

Figure 4: a) The central tree on the genetic monitoring plot is marked with multiple bands to differentiate it from other trees (an example of a European beech FGM plot); b) numbers are painted on selected trees so that they point away from the central tree. Image depicts a Silver fir (*Abies alba* Mill.) forest genetic monitoring plot in Bavaria.

6.2 Establishment of natural regeneration subplots

The establishment of natural regeneration (NR) subplots should be carried out during germination after a strong or massive fructification event.

Natural regeneration centres from the last mast year should be surveyed in the field and their locations logged (GPS coordinates, number of the tree which is next to an NR centre). From all logged regeneration centres, 20 should be chosen randomly for plot installation. If 20 or fewer natural regeneration centres are present, all should be used.

Inside each selected natural regeneration centre a 1m^2 plot is to be installed and marked with metal rods. The metal rods should be driven into the ground at each corner of the subplot as deep as possible to prevent them from being removed by animals. The tips of the metal rods should be painted to aid their visibility.

6.3 Plot maintenance

6.3.1 General maintenance

Tree markings and subplot markings must be checked periodically (every two years) and repaired 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 (preferably in the outer circle) of the FGM plot is to be selected. The replacement tree is marked with the next available number higher than 50, i.e. 51, 52, 53, etc. to positively differentiate it from the original 50 selected trees.

If the crown is damaged due to, for example, wind break, ice or snow break but continues to fructify, the tree is kept for 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

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 is 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 recording of background information.

Table 2: List of verifiers and background information with short description and observation frequency to be recorded during field work at the black pine monitoring plots.

	Name	Basic level	Standard level	Advanced level
Verifiers	Mortality / survival	Adult trees: Counting of the remaining marked trees every 10 years and after every extreme weather event/disturbance	Same as basic level	Same as basic level
		Natural regeneration: /	Counting of remaining seedlings on the natural regeneration subplots, twice per decade	Same as standard level
	Flowering	Stand-level estimate, every year	Individual tree level observation, during two major flowering events per decade, ideally equally spaced *	Individual tree level observation, during two major flowering events per decade, ideally equally spaced *
	Fructification	Stand-level estimate, every year	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, the same years as the assessment of flowering at the advanced level, regardless of the fructification intensity * Seeds are also collected for laboratory analyses every assessed fructification event
	Natural regeneration abundance	Stand-level estimate, every year	Counting of seedlings in the 1st and 6th years after every assessed fructification event	Counting of seedlings in the 1st, 6th, 11th, and 16th years after every assessed fructification event
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, 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.

7.1 Protocols for recording of verifiers

7.1.1 Mortality / survival

Mortality describes the mortality of adult trees and natural regeneration. 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 is estimated by counting the remaining alive marked trees 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.

7.1.1.2 Natural regeneration: Standard and advanced levels

Mortality of natural regeneration is calculated from the verifier Natural regeneration abundance. Mortality is the difference between the initial number of NR plants and the plants remaining alive at the time of the next counting. For each round of assessment, the NR is counted first in the year of germination and then again after 5 years at the standard level, while at the advanced level the counting is also performed after 10 and 15 years. Assessment of NR abundance is carried out twice per decade, ideally approximately every five years.

7.1.2 Flowering

This verifier describes the flowering intensity and the proportion of trees thus affected. It can usually be recorded from late April till early June.

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, expressed as the average proportion of the crown flowering, 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	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). On average, two visits to the plot are needed; the first one early enough to observe the early stages of flowering and the second one when flowering is in full progress.

Three scores are provided for each tree: female and male flowering stages [5], and the proportion of the crown flowering. The proportion of the crown flowering refers to the total number of flowers (male + female) on the tree. For graphical representation of the flowering stages, see Figure 5.

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 the next major flowering event. Basic level observations are used to identify major flowering and fructification events.

Code	Female conelets phenological stages
1	Female flowering buds clearly visible on the top of the shoot but scales are completely covering the female conelet.
2	The apex of the cylindrical conelet is opened and the first ovuliferous scales appear.
3	The scales of the female conelet are separated and almost form right angles with the conelet axis (receptivity 100%).
4	The scales of the conelet are closed.

Code	Male strobili phenological stages
1	Male strobili are developing, but still closed in integuments.
2	Microsporangia are not tightly packed, and green to yellow liquid emerges from the strobili when pressed.
3	Yellow strobili shedding their pollen.

Code	Proportion of the crown flowering (%; male and female flowering together)
1	0 – 10
2	> 10 – 30
3	> 30 – 60
4	> 60 – 90
5	> 90

The background information Flowering Synchronisation can be estimated from the scores for female and male flowering recorded by this verifier.

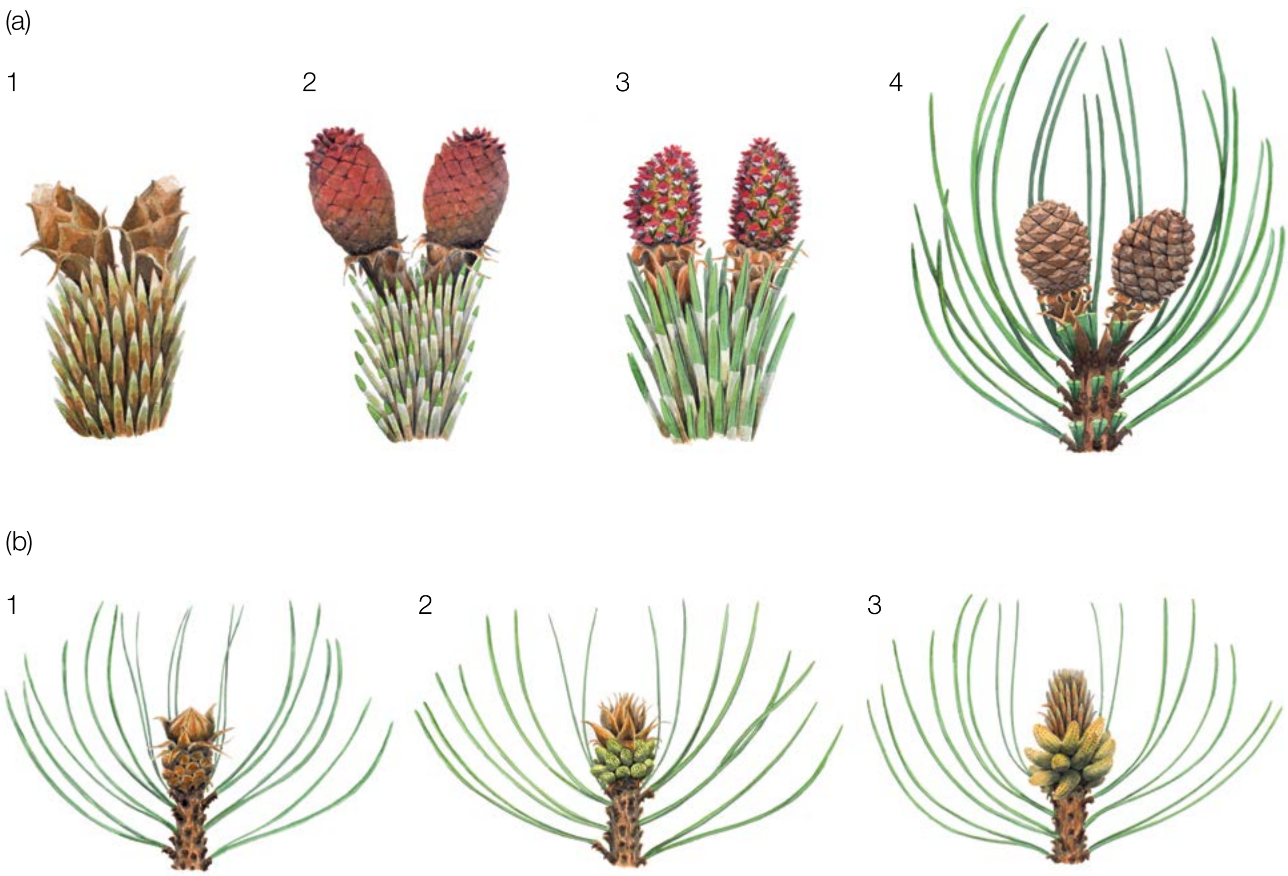

Figure 5: Picture guide for description of *Pinus nigra* female conelets (a) and male strobili (b) stages for the advanced level verifier Flowering.

7.1.3 Fructification

The verifier indicates the presence of fructification and its abundance for *Pinus nigra*. Data for this verifier should be collected during the fructification period of *Pinus nigra,* and when cones are mature, i.e. from September to November. It should be mentioned here that the cones of the species mature the second autumn after the flowering.

7.1.3.1 Basic level

This verifier is recorded every year at the stand level. The estimate of average condition is provided after a walk throughout the monitoring plot. Two scores are given, one for fructification intensity and one for the proportion of fructifying trees in the stand.

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

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

7.1.3.2. Standard level

This verifier should be recorded the second autumn (September/November) after the assessment of the flowering at the standard level (regardless of the fructification intensity). It is recorded at an individual tree level on all 50 monitored trees. Recording is carried out before the mature cones shed their seeds and 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 when the next major flowering event and the subsequent fructification occurs, 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 occurring at the basic level when fructification intensity is strong or massive (code 4 or 5), and the proportion of trees with the given fructification intensity is above 60% (code 4 or 5).

Code	Fructification intensity	Proportion of the crown fructifying (%)
1	No fructification: No or only occasional fruits appearing on a tree.	0 – 10
2	Weak fructification: Some fruits 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

7.1.3.3 Advanced level

This verifier is recorded at an individual tree level on all 50 monitored trees two years (the second autumn) after the assessment of flowering at the advanced level, regardless of the fructification intensity. Recording is carried out before cones are open and seed is dispersed. One score is provided for each tree. Simultaneously, seed is collected 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 two years after 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 level fructification intensity is strong or massive (code 4 or 5) and the proportion of trees with the given fructification intensity is above 60% (code 4 or 5).

The verifier is recorded by counting cones using binoculars. The average of three rounds of counting is reported. Each round of counting consists of the number of cones that the observer counts in 30 seconds. For all trees, the same part of the crown should be observed for cone counting. 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 parts for cone counting.

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

Number of cones 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 abundance

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

7.1.4.1. Basic level

This verifier is recorded at the stand level every year in the autumn. Expert opinion is used for estimation considering the situation over the whole monitoring plot. Two values should be recorded, one for new natural regeneration (current-year seedlings) and one for established regeneration (saplings that are older than one year). Since full seed crops (mast years) for *Pinus nigra* usually occur every 3 to 5 years, the establishment of new NR should be estimated the summer/autumn following the mast year.

Code	Description: new regeneration (current-year seedlings)
1a	There is no or very little new natural regeneration on the monitoring plot.
2a	New regeneration is present in sufficient numbers 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.

7.1.4.2 Standard level

This verifier is recorded by counting of plants/seedlings the 1st autumn after every assessed fructification event (the year of the fructification event is regarded as year 0) and 6th autumn after the fructification event.

Counting of seedlings:

After the establishment of NR subplots all *Pinus nigra* seedlings present at each of the 20 NR subplots must be counted. Any older black pine saplings that are growing on the NR subplot should not be included. During the next counting round, only saplings of the appropriate age must be counted – i.e. in the 6th year, 5-year old saplings.

Number of seedlings counted on a subplot
X

Mortality/survival of natural regeneration is calculated from the values recorded for this verifier.

For subplot establishment see 6.2 Establishment of natural regeneration subplots.

7.1.4.3. Advanced level

This verifier is recorded by counting seedlings at each one of the 20 NR subplots in the 1st autumn after the major fructification event (the year of the fructification event is regarded as year 0) and the 6th, 11th, and 16th autumns after the fructification event. The next round of monitoring of natural regeneration abundance (establishment of new 20 NR subplots and assessment of NR abundance) is carried out after the first fructification event at least 5 years after the previous major fructification event (see Table 3 for a representation of the NR abundance assessment timeline). Assessment of NR abundance from one or two major fructification events per monitoring interval is expected.

Table 3: Timeline of natural regeneration abundance (NR) assessment. In this example, the first major fructification event takes place in the second year of the monitoring decade, and the second assessed fructification event five years later, i.e. in the 7th year of the monitoring. Because major fructification events occur every 3 – 5 years for *Pinus nigra*, the interval between any two consecutive major fructification events can vary accordingly. Twenty new NR subplots are established after each assessed fructification event. Monitoring of NR abundance on each set of 20 NR subplots is carried out every five years. The fructification events corresponding to the assessed NR and timelines of the assessment activities are shaded in the same colour. After the final round of counting of seedlings, monitoring of NR abundance on the respective set of NR subplots is stopped and the respective NR subplots disestablished. S – standard level; A – advanced level.

Year of monitoring	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Fructification event		•					•							•					•				
NR assessment from the 1st assessed fructification event [yrs]		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
NR subplots establishment			SA																				
NR abundance counting			SA					SA					A					A					
NR assessment from the 2nd assessed fructification event [yrs]							0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
NR subplots establishment								SA															
NR abundance counting								SA					SA					A					A

Mortality/survival of natural regeneration is calculated from the values recorded for this verifier. For subplot establishment see 6.2 Establishment of natural regeneration subplots and for counting 7.1.4.2 Standard level.

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 at an individual tree level on all the 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, all of them should be measured and their average value should be estimated (try to avoid individuals with many thin trunks). Keep a note indicating that the tree is a multi-trunk one in the notes section. If the tree is leaning, the DBH should be measured perpendicular to the trunk. DBH can be measured in two ways:

1) By using a calliper, in which case you need to measure two perpendicular diameters and take the average.
2) By measuring the circumference of the tree and computing 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 at an individual tree level on all the 50 monitored trees, every 10 years. Height is measured from the ground to the tallest part of the crown, ideally by using a clinometer or a 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 reason for this in the notes.

7.2.3 Budburst

Budburst describes the process of budbursting (flushing). In *Pinus nigra*, budbursting starts a bit later than flowering. Recording of this parameter is only carried out at the standard and advanced levels. Data of this background information should be collected in April – May, until all monitored trees have reached the stage of fully developed needles.

7.2.3.1 Standard level

At standard level, budburst is recorded at an individual tree level on all the 50 monitored trees, every 5 years. We are looking for the initiation of budbursting (stage 2) and the end of budbursting (stage 5). The observations are terminated when all the trees have reached stage 5. Usually, six visits will be needed. For each tree, two estimates are given: stage of budbursting and the proportion of the crown budbursting. For a graphical representation of budbursting stages please see Figure 6.

Code	Stage of budbursting (Simplified stages [5])
1	Dormant buds
2	Start of elongation
3	Significant elongation of terminal bud
4	Needles emerge from transparent envelopes
5	The two needles of the same brachyblast are clearly distinct

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

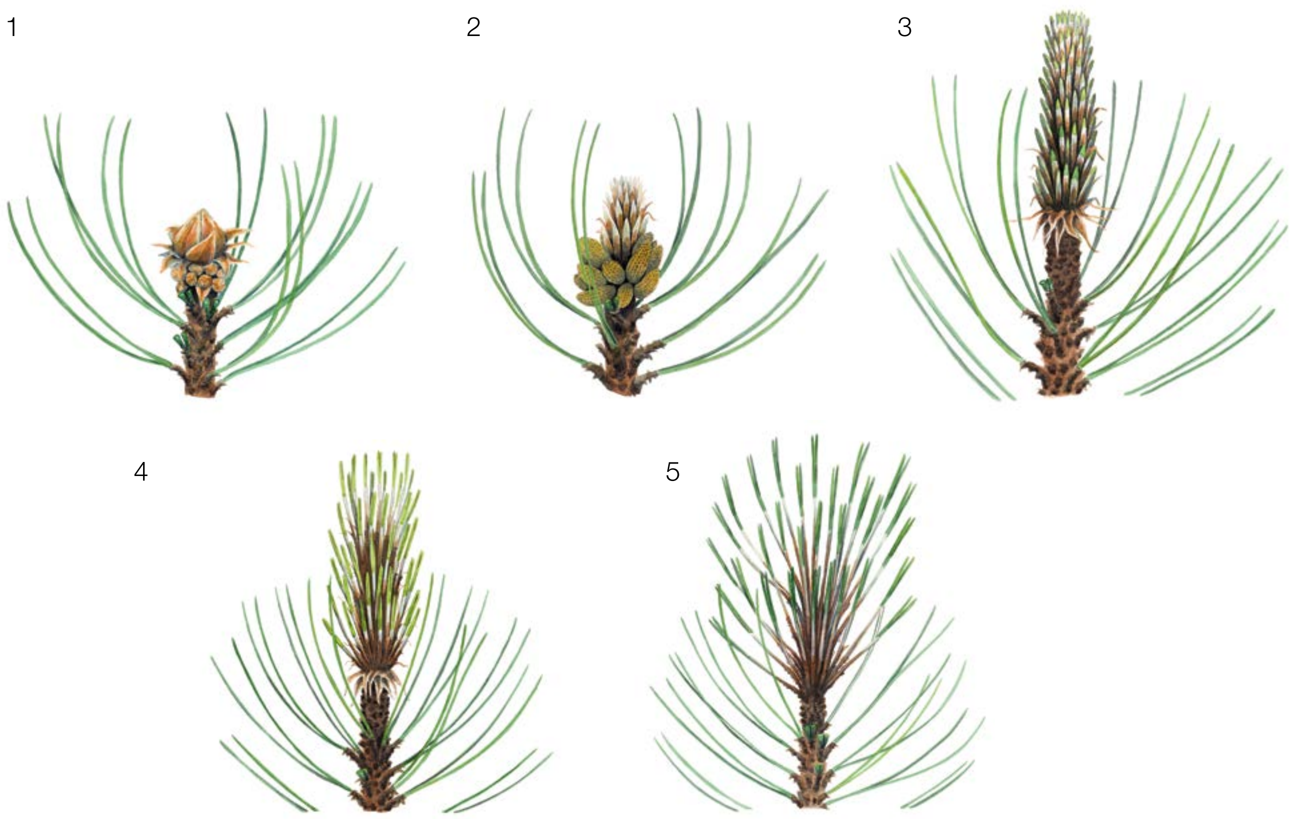

Figure 6: Picture guide for description of budburst (flushing) for the basic, standard and advanced level background information Budburst.

7.2.3.2 Advanced level

At the advanced level, budburst is recorded at an individual tree level on all the 50 monitored trees, every year in the same way as at the standard level. For details see 7.2.3.1 Standard level.

7.2.4 Flowering synchronisation

Flowering synchronisation is monitored only at the advanced level, and is based on the data collected for the verifier Flowering. It is used to determine whether the male and female flowering periods overlap for the trees on the monitoring plot [19].

7.2.4.1 Advanced level

Flowering synchronisation is recorded at an individual tree level, and for all the 50 monitored trees, during each assessed major flowering event. From that flowering event the seed that will be collected the second autumn after flowering will be produced.

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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