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SEPARATE

Guidelines for genetic monitoring of

# Silver fir
## (*Abies alba* Mill.)
## and
# King Boris fir
## (*Abies borisii-regis* Mattf.)

Separate is a part of publication

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Guidelines for genetic monitoring of

# 9.2.1 Silver fir (*Abies alba* Mill.) and King Boris fir (*Abies borisii-regis* Mattf.)

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# 1 Executive summary

Silver fir (*Abies alba* Mill.) and King Boris fir (*Abies borisii-regis* Mattf.) are wind-pollinated, monoecious, generally outcrossing conifer tree species, which belong to the *Abies* genus. Silver fir is one of the most important forest tree species from an economic and ecological point of view in several European countries. King Boris fir is an important natural hybrid between *Abies alba* and *Abies cephalonica* Loudon, growing mainly in Greece. Both species are facing many threats and challenges due to climate change, and therefore they are both considered for forest genetic monitoring.

The guidelines briefly describe Silver fir and King Boris fir, their reproduction system, environmental requirements and threats. They provide guidance on how to establish and maintain a forest genetic monitoring plot and on recording all field level verifiers and phenotypic data at the basic, standard, and advanced monitoring levels.


**Figure 1:** Silver fir (*Abies alba*) habitus (a); a seedling of Silver fir/King Boris fir (b); a branch with needles of the King Boris fir (c) and the Silver fir (d).

# 2 Species description

Silver fir and King Boris fir (Figure 1) are monecious conifer tree species, which in optimal conditions can reach more than 50 m (60 m) in height and more than 1.5 m (2.0 m) in diameter (DBH) [1, 4, 16, 28]. Due to the low growth of the peak shoot the older trees lose their conical appearance and become oval at the top. The wood has no resin or coloured core. The branches stand in whorls and not hanging, but mostly horizontally laid, more or less flat. The bark is rough and uncracked, up to the age of 50 years old. In older age the bark gains the form of squared shells of cork, which remain attached to the tree and are hard to separate [1, 4, 7, 26, 27, 28]. The needles of silver fir are dark green and glossy on their upper side, while the lower side has two silver green waxy bands of 6-8 rows of stomata [16, 28].

## 3 Reproduction

*Abies alba* and *Abies borisii-regis* are wind-pollinated, monoecious, generally outcrossing species with a chromosome number of 2n=24. Seeds of both species are wind-dispersed and female flowers are located on the upper top twigs, in the form of small cones. Male flowers usually are located a bit lower in the crown, in the armpit of needles, in the form of catkins. Male flowers are roughly 2 cm long with two pollen bags. Silver fir flowers in spring, from April to June, depending on altitude and latitude [1, 4, 7, 26, 27, 28].

Firs are long-lived species, attaining reproductive ability the earliest at the age of 20 years and averaging at the age of 60 years [14]. Female flowers are in the form of cones, in young age dark green, egg-shaped, about 2 cm long and upright. The mature cone is yellowish to dark brown, cylindrical, up to 16 cm long and up to 5 cm wide. Cones always stand upright on twigs, decaying in the same year, and in October shells with seeds fall, with a naked axis (spindle of a cone) is left on the branch. Winged seeds disperse by wind. While the tree is young, it fructifies every two years, but old trees and those at higher altitudes fructify less often, every three years or more [1, 4, 7, 26, 27, 28]. However, some trees may fructify every year (LIFEGENMON observations in *Abies alba* FGM plot in southern Germany).

Despite high amounts of pollen production, silver fir is considered a weak seed producer, because of few buds developing into female flowers. Moreover, insect attacks, late frost, usually in late May and early June, depending on the altitude [8, 10], and inadequate pollination decrease seed production drastically [6, 15, 17, 18, 19, 20, 23, 24, 25]. The whole cycle from flowering to seed maturation and dispersion takes place in one year. The period from flowering and pollination to the maturation of the seed lasts from 90 to 120 days [3, 9, 12, 13, 29]. Silver fir seed mature and disperse between September and November.

## 4 Environment

Silver fir is distributed in Central Europe and in some parts of Eastern and Sothern Europe [4, 26, 27, 28]. Distribution of King Boris fir is limited to the southern part of the Balkan Peninsula. Silver fir is a species of moderate continental climate softened by the ocean, and in contrast to other Mediterranean *Abies* species it prefers cooler and moister conditions. Silver fir tolerates a wide variety of soil types with different nutrient content and alkalinity conditions, except compact and hydromorphic soils [16]. Deep and moist but not too wet soils are preferred, with a pH from acid to neutral. Silver fir is a highly shade tolerant tree species and can remain in a densely shaded selective forest for a very long time [16, 28]. Silver fir can form pure stands, but can usually be found in the upper tree limit mixed with Norway spruce (*Picea abies* (L.) H. Karst.) or Scots pine (*Pinus sylvestris* L.) and at lower altitudes it can grow with European beech (*Fagus sylvatica* L.) [1, 16, 28, 4 and references therein].

## 5 Threats

Silver firs are very sensitive to temperature requirements, because offspring suffer from late spring frosts [21]. Young silver fir plants, up to three years, are very sensitive to drought, and if drought lasts for a longer time then the young plants cannot survive [1, 4, 22]. In addition, natural regeneration of silver fir is very susceptible to animal browsing. Silver fir is also sensitive to forest fires, air pollution, especially to $SO_2$ exposure during winter [16 and references therein]. Because of the changing climate silver firs are more susceptible to diseases and pests, e.g. mistletoe and bark beetles have already damaged silver fir in the Mediterranean, especially in those areas where droughts occur more frequently [16 and references therein]. Insects like *Ips typographus* L., *Cinaria pectinatae* Nördlinger and *Epinotia nigricana* Herrich-Schäffer affect the bark and buds of silver fir. The fungi *Armillaria mellea* (Vahl) P. Kumm agg. and *Heterobasidion annosum* (Fr.) Bref. are responsible for butt rot and windthrow [16 and references therein].

# 6 Plot establishment and maintenance

Silver fir is a stand forming tree species which can form pure or mixed forest stands with Norway spruce, Scots pine, European beech and other tree species [1, 28, 4 and references therein]. Therefore, a regular FGM scheme, as for other stand forming tree species e.g. *Fagus sylvatica*, can be applied for genetic monitoring of *Abies alba* and *Abies borisii-regis.* Key steps for successful FGM plot establishment for *Abies alba* and *Abies borisii-regis* are: FGM plot selection according to the described criteria (e.g. high priority should be given to forest stands for which high data density and precise plot documentation is already available) [2].

However, FGM of *Abies borisii-regis* due to geographically disjunct distribution, biology (e.g. hybridisation) and the threats (e.g. climate change, pests and diseases), can be more challenging and develop the need for larger FGM plots. Therefore, the size and design of the FGM plot should be flexible, depending on the local specificities, but it is not recommended to be bigger than 10 ha for practical purposes.

A forest genetic monitoring plot consists of 50 reproducing trees with the minimum distance of 30 m between any two trees. If a tree is flowering, it is regarded as a reproducing tree. DBH and social class can be used as proxies to identify a reproducing tree if the plot is being established outside of the flowering season, relying on the expertise of the local forester. During plot installation, trees should be labelled and coordinates of all trees taken. At the same time DBH can be measured and samples for DNA extraction taken.

In addition, the presence of sufficient density of natural regeneration must be found within the FGM area.

Equipment needed:

- a device for distance measurement (a pair of range-finding binoculars is recommended)
- a compass,
- a paint with a brush or spray for marking trees,
- a tree calliper for DBH measurements, and
- a GPS device that is precise enough and allows saving trees' coordinates.

## 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 2a):

- Random selection of a point (green dot) on a map along the forest road or path, which runs along the stand,
- Drawing a line that is approximately perpendicular to the road from the randomly selected point on a road,
- Random selection of one point on the perpendicular line (red dot) – this point represents the centre of the forest genetic monitoring 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.

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

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

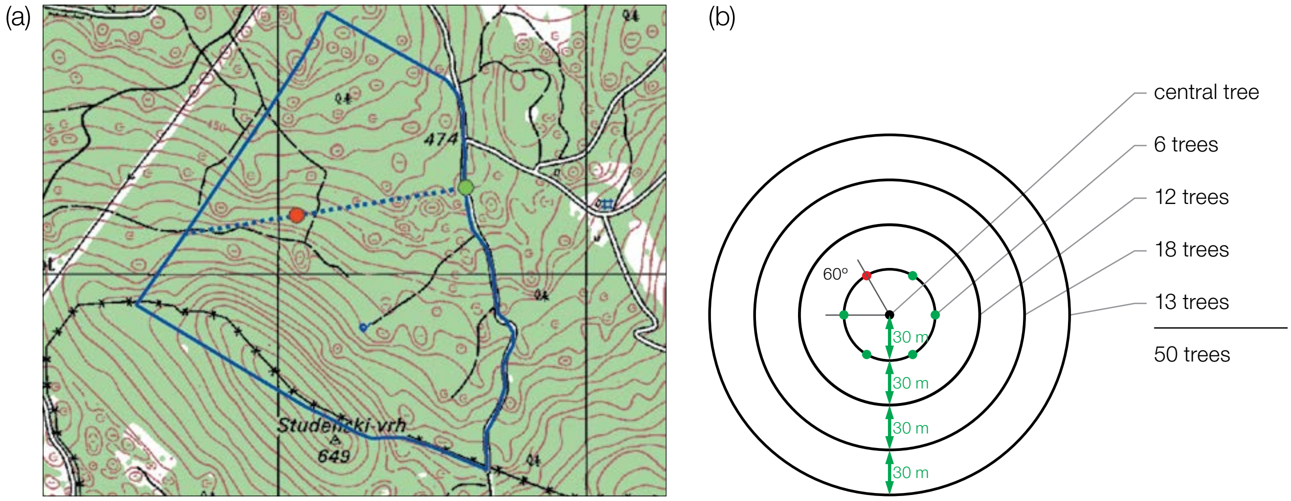


**Figure 2:** Random selection of the centre of the forest genetic monitoring plot (a); selection of trees in concentric circles around previously selected central tree with an increasing radius of 30 m (b).

### 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 2b). 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 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 six (6), 12 and 18 trees in the inner three circles (Figure 2b), 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 |

### 6.1.3 Labelling of trees

All 50 selected trees (DBH ≥ 15cm) must be marked with a corresponding number (from 1 to 50) and preferably a band painted around the trunk to aid the visibility of the trees from all directions. Coordinates (X/Y) of each tree must be taken (GPS needed). Tree numbers given during adult tree selection must be maintained over the entire monitoring period. The central tree (number 1) can be marked with two or more bands to differentiate it from other trees (Figure 3a). It is recommended to paint the number on the side of the tree that is pointing away from the central tree, as this helps locating the central tree, particularly from the outer rings of the plot (Figure 3b). In some cases, it helps to mark the trees on the side pointing away from paths or roads in order to avoid confusing or drawing the attention of the people seeking recreation in the forest.


**Figure 3.** The central tree on the genetic monitoring plot is marked with multiple bands to differentiate it from other trees as done on a European beech FGM plot in Slovenia (a); numbers are painted on selected trees so that they point away from the central tree (b).

## 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 a 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. 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 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 (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, windbreak, 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

On the monitoring plot, verifiers and background information are periodically recorded. 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 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 *Abies* spp. 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 for every assessed fructification event |
| | Natural regeneration abundance | Stand-level estimate, every year | Counting of seedlings according to the protocol in the 1st and 6th years after every assessed fructification event | Counting of seedlings according to the protocol in the 1st, 6th, 11th, 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 according to the protocol, every 5 years | Individual tree level observation according to the protocol, 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 mortality of *Abies* spp. 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 initial number of marked trees and the trees remaining alive of the original 50.

#### 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 the flowering intensity and the proportion of trees thus affected. It can usually be recorded in April to May in central Europe.

#### 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 the 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 *Abies* spp. 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. Proportion of the crown flowering refers to the total amount of flowers (male + female) on the tree. For a graphical representation of flowering stages see Figure 4.

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 flower stage |
|---|---|
| 1 | Small conelets visible (1-2 cm) |
| 2 | Conelet elongation started |
| 3 | Cone colour changed from green to brownish (colour monitoring) |

| Code | Male conelet phenology |
|---|---|
| 1 | Micro-sporophylles are starting to extend their size but are still closed and situated very close to the branch (colour – green/brown/dark red/reddish brown) |
| 2 | Pollen bags are extended/swollen, ripened – release of pollen (colour – yellow/dark red/brown/reddish brown) |
| 3 | Release of pollen concluded, bags still hanging on the branch but empty (colour – brown/dark red/reddish brown) |

| 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 |

Background information on flowering synchronisation can be estimated from the scores for female and male flowering recorded by this verifier.

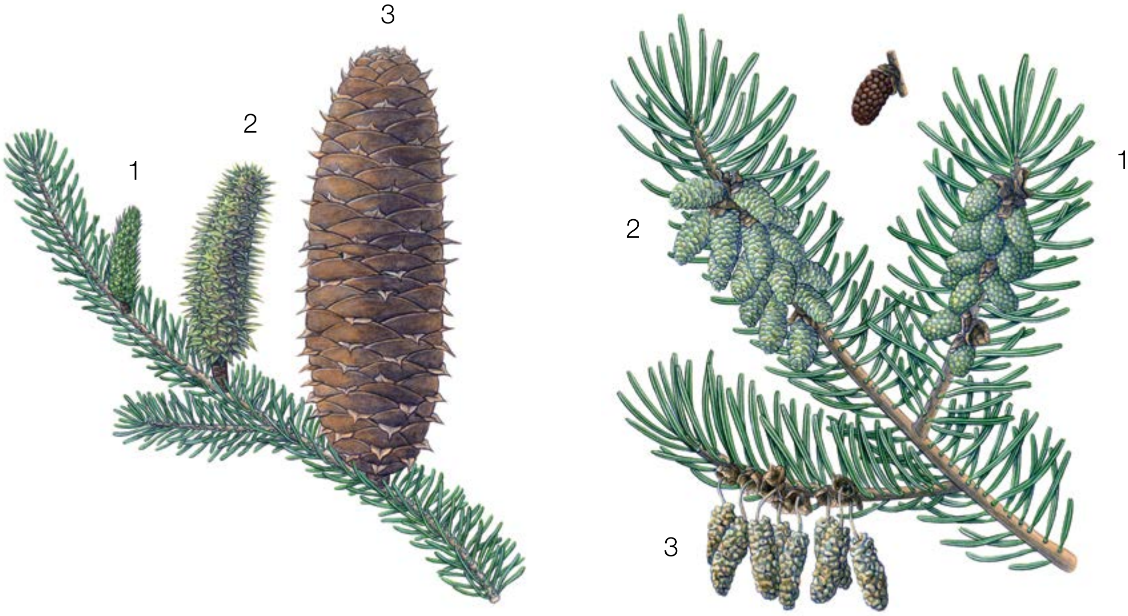


**Figure 4:** Picture guide for description of female (left) and male flowering (right) stages for the advanced level verifier Flowering.

### 7.1.3. Fructification

This verifier describes the presence of fructification and its abundance for *Abies* spp. Data for this verifier should be collected during fructification of *Abies* spp., which usually occurs in August/September in central Europe.

#### 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 fruits appearing on trees | 0 – 10 |
| 2 | Weak fructification: Some fruits appearing on trees | > 10 – 30 |
| 3 | Moderate fructification: Moderate number of fruits appearing on trees | > 30 – 60 |
| 4 | Strong fructification: Abundant number of fruits appearing on trees | > 60 – 90 |
| 5 | Massive: Huge number of fruits 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 is recorded during the same years as 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 fruits start falling. One score is provided for each tree.

Ideally, one major fructification event should be captured following observations of major flowering events each 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 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).

| 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 number of fruits appearing on a tree. | > 30 – 60 |
| 4 | Strong fructification: Abundant number of fruits appearing on a tree. | > 60 – 90 |
| 5 | Massive: Huge number of fruits 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 during the same years as the assessment of flowering at the advanced level, regardless of the fructification intensity. Recording is carried out before fruits start falling. 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 each 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 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 is able to count 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 parts 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 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 *Abies* spp. fructifies every three to five years, the establishment of new NR should be estimated next summer/autumn after 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 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 |

#### 7.1.4.2 Standard level

*Abies* spp. seed dormancy lasts one winter, therefore, this verifier is recorded by counting of plants/seedlings starts in the 1st autumn after the 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 *Abies* seedlings present at each of the 20 NR subplots must be counted. Any older Silver fir/King Boris fir saplings that are present on the NR subplot must not be included. During the next counting round, only saplings of the appropriate age must be counted – in the 6th year, five-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.

#### 6.1.4.3 Advanced level

*Abies* spp. seed dormancy lasts one winter, therefore this verifier is recorded by counting seedlings at each 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 in 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 five years after the previous major fructification event (see Table 3 for representation of the NR abundance assessment timeline). Assessment of NR abundance from one or two major fructification events is expected each monitoring interval.

**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 seventh year of the monitoring. Because major fructification events occur every three to five years for *Abies* spp., 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 on an individual tree level for 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, measure all of them and record the average (but try to avoid trees with many small trunks). Note that the tree is a multi-trunk one in the notes. 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 cases you would 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 meters, rounded to the closest full meter. 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). In silver fir, 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 in central Europe, until all monitored trees have reached fully developed needles.

#### 7.2.3.1 Standard level

At standard level, budburst is recorded on an individual tree level on all 50 monitored trees every five years. We are looking for the initiation of budbursting (stage 2) and the end of budbursting (stage 4). The observations cease when all the trees have reached stage 4. Usually, six visits will be needed. For each tree, two estimates are given: budbursting stage and proportion of the crown budbursting. For a graphical representation of budbursting stages, see Figure 5.

| Code | Stage of budbursting (Simplified stages [5]) |
|---|---|
| 1 | Buds enclosed by needles and not visible unless the needles are parted |
| 2 | Buds elongation, bud scales and membrane visibly abscised |
| 3 | Elongating brush of soft needles has emerged |
| 4 | Soft shoots with developed needles |

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

#### 7.2.3.2 Advanced level

At advanced level, budburst is recorded on an individual tree level on all 50 monitored trees every year. For the values (stage of budbursting and the proportion of crown affected) see 7.2.3.1 Standard level.

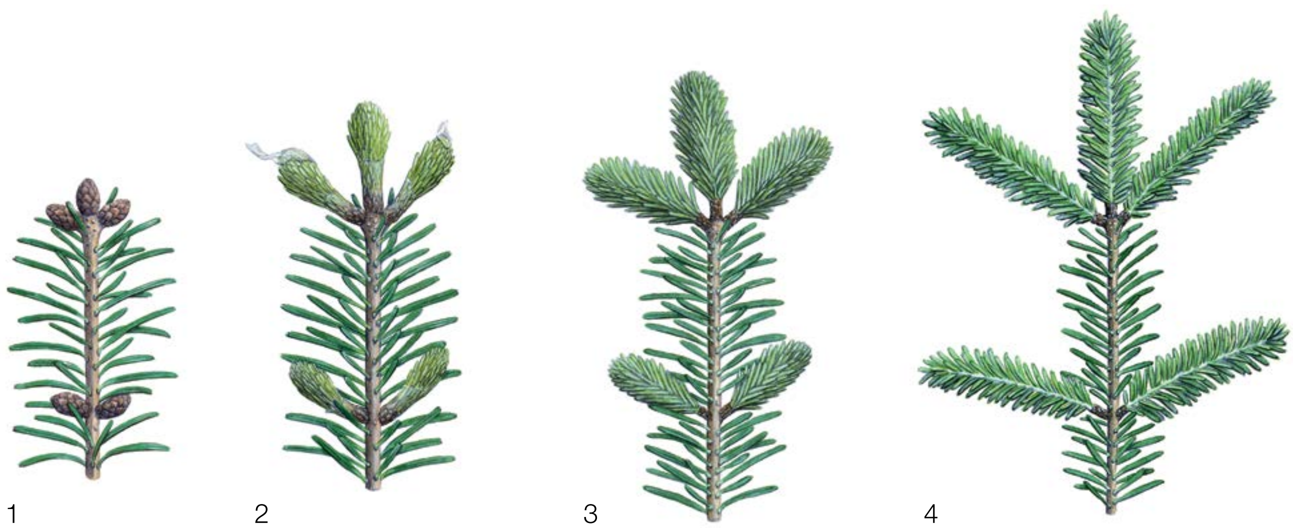


**Figure 5:** Picture guide for description of budburst (flushing) for the basic, standard and advanced levels, background information Budburst.

### 7.2.4 Flowering synchronisation

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

#### 7.2.4.1 Advanced level

Flowering synchronisation is recorded on an individual tree level on all 50 monitored trees, during each assessed major flowering event, in the same years as when seed is collected (the same as Flowering at the advanced level).

**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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