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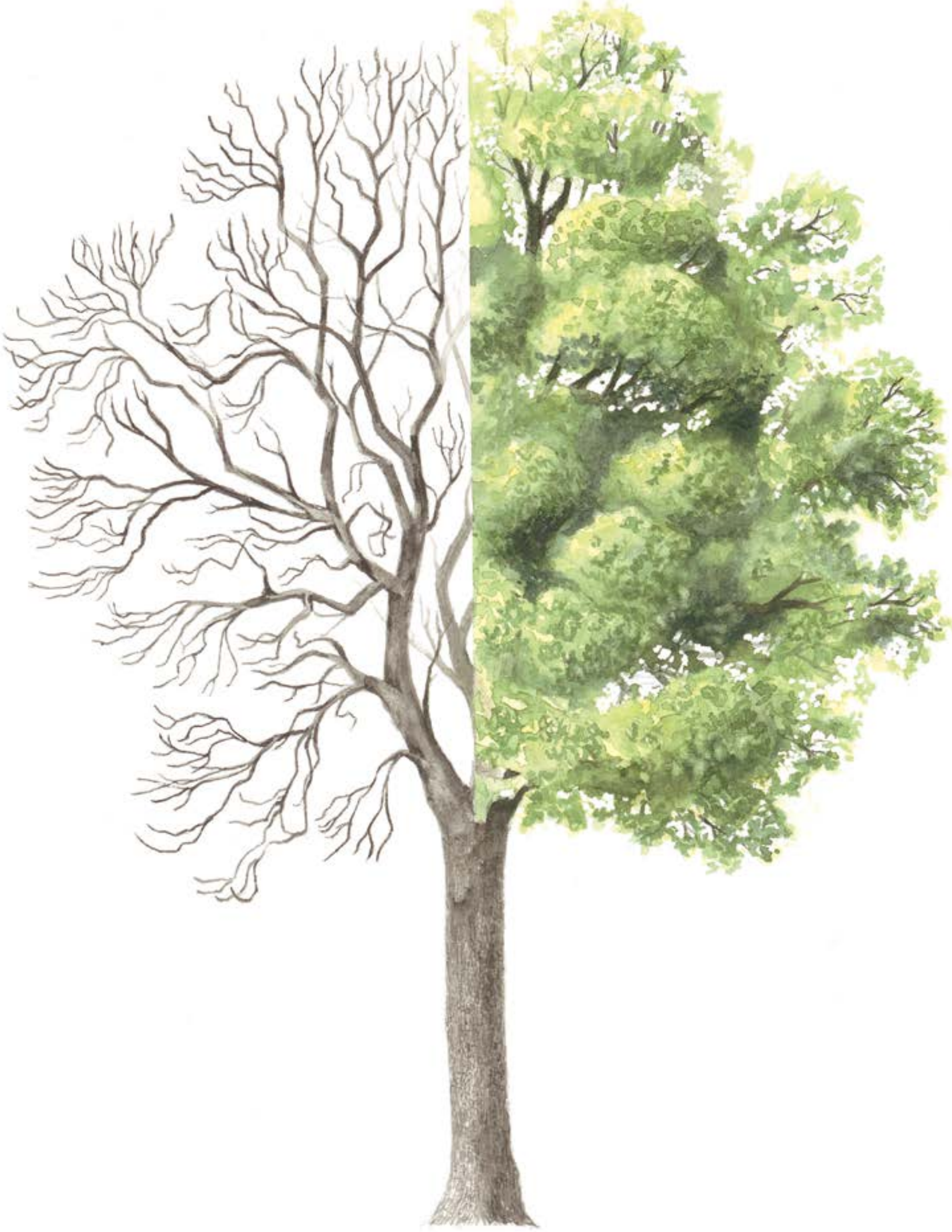
SEPARATE

Guidelines for genetic monitoring of

Common ash
(*Fraxinus excelsior* L.)



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Guidelines for genetic monitoring of

9.2.3 Common ash (*Fraxinus excelsior* L.)

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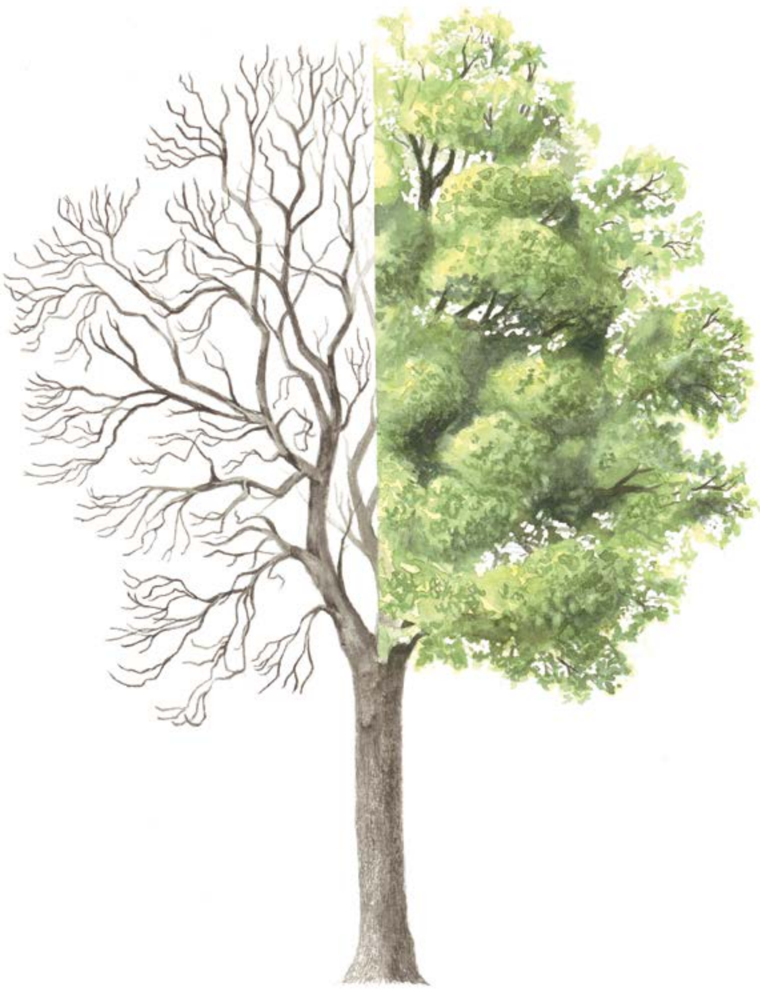

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1 Executive summary

Common ash (*Fraxinus excelsior* L.) is a polygamous, deciduous tree species present throughout Europe except in the driest Mediterranean areas. Common ash can form pure stands but is more commonly found growing in smaller groups of trees in mixed stands akin to species with scattered distribution. It is closely related to narrow leaved ash (*F. angustifolia* Vahl.) with which it hybridises. With its high ecological importance and utility in the timber industry, this species that is strongly threatened by the ash dieback is a prime candidate for genetic monitoring.

These guidelines briefly describe the common ash, its reproduction, environment and threats. They provide guidance on establishing a genetic monitoring plot and on recording all field level verifiers and background information.

2 Species description

Common ash (Figure 1) is a deciduous tree reaching heights up to 40 m when 90-120 years old [1]. The crown is irregular with massive branches, elongated in forest stands [1]. The bark is pale brown to grey, which fissures as the tree ages [2]. In the winter, it is easily identified by smooth twigs that have distinctively black, velvety leaf buds arranged opposite each other. Leaves are pinnately compound, typically comprising 7-13 oval leaflets with long tips including an additional singular 'terminal' leaflet at the end [2, 3] (Figure 2a). These leaves are up to 35 cm long [2], light green on the bottom side and green-grey on the upper one.

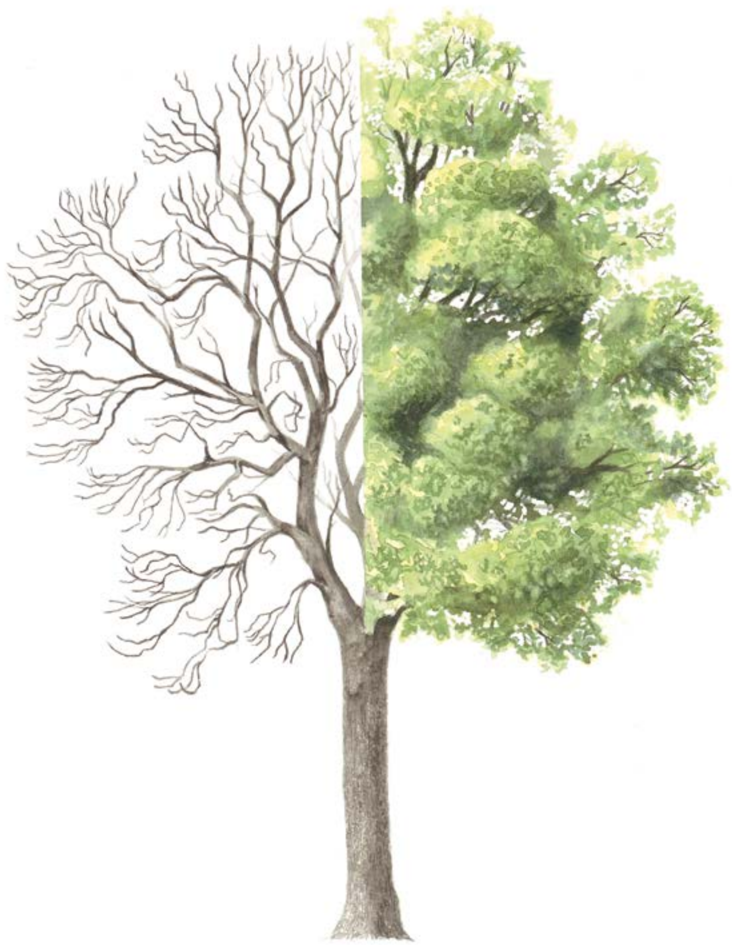

Figure 1: Common ash (*F. excelsior*) habitus.

Common ash is closely related to *F. angustifolia* Inflorescence or fructification type is the most reliable characteristic to distinguish them (Figure 2); common ash has a branched inflorescence while *F. angustifolia* has an unbranched simple raceme [3]. However, some *F. excelsior* trees have mixed inflorescences with hermaphrodite flowers only on the main axis of inflorescences and male flowers only on the secondary branches of inflorescences, and these can be misidentified as after the fall of male flowers their raceme may appear as an unbranched raceme of *F. angustifolia* [3]. Hybrids have been reported in areas where the two species grow together [2, 3].

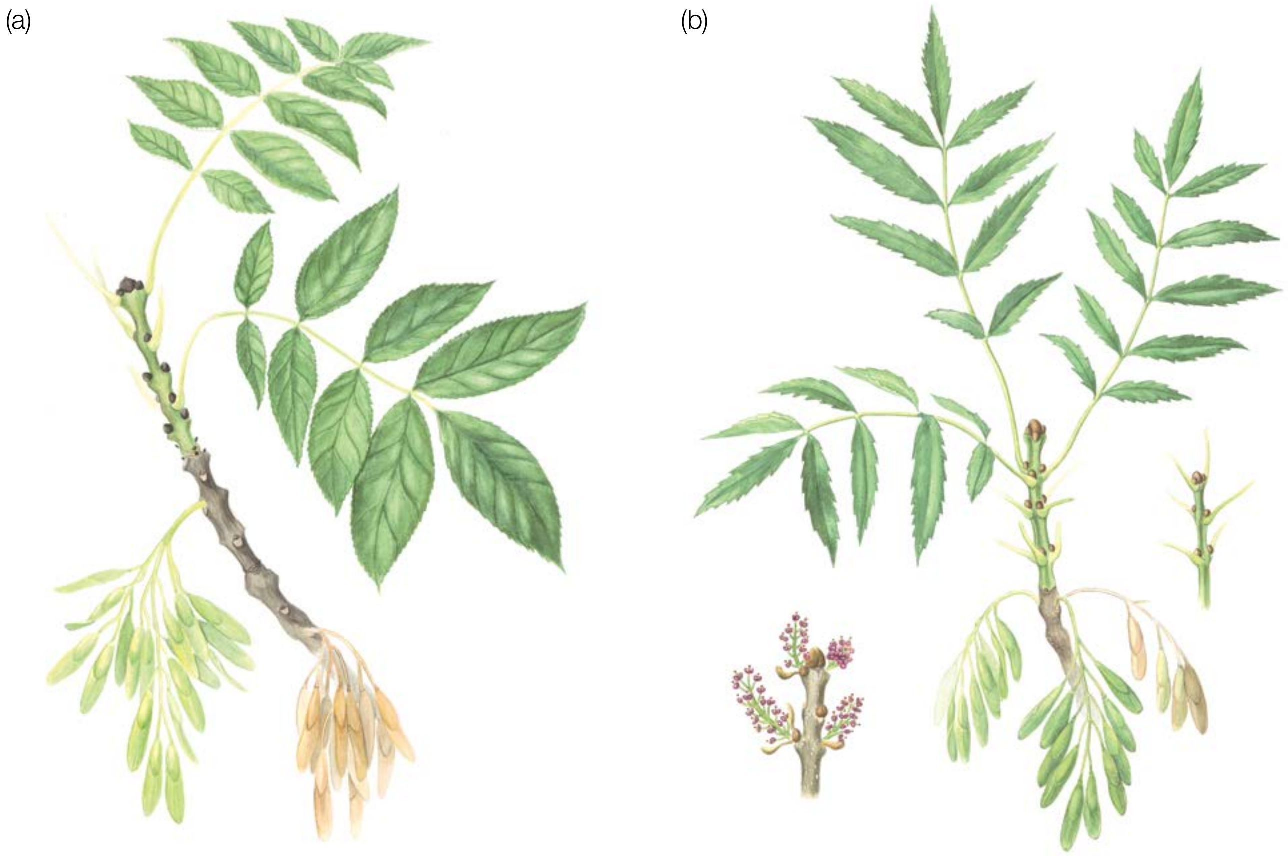

Figure 2: Morphological signs for differentiating *F. excelsior* (a) from *F. angustifolia* (b).

3 Reproduction

Common ash is polygamous; it can develop only male or female inflorescences on a single tree, or unisexual inflorescences with only male and female inflorescences carried separately on the same tree, or even hermaphrodite inflorescences [1, 2, 3]. It is self-fertile [3]. However, selfed seeds may not survive because of inbreeding depression, making the species possibly functionally dioecious [3]. Both male and female inflorescences are purple and appear in March to April in central Europe, before the leaves in the spring, growing in spiked clusters at the tips of twigs. Leaves flush after flowering has finished, on the shoots that emerge from the terminal buds. The start of budbursting (flushing) varies from population to population and year to year; flowering and budbursting are earlier when preceded by a warm winter [3].

Once the female inflorescences have been pollinated by wind, they develop into clearly visible winged fruits – samaras – in late summer and autumn. They fall from the trees in winter and early spring, and are mainly dispersed by wind [1, 2, 3]. Flowering starts at 15-20 years on single trees and at around 30 years within stands at irregular intervals [1]. Seeds are usually dormant for two winters before germinating, but also longer, for up to six years on dry or high locations [2, 3].

Common ash exhibits intermediate properties between a pioneer and climax species. Dispersal and natural regeneration are efficient; however, its competition ability is only strong when the ecological requirements are met [2, 3]. Vegetative regeneration is strong after coppicing [3].

3.1 Identification of tree's sex

Male trees are trees on which most inflorescences are male. This category can be subdivided into purely male trees (with only male inflorescences) and those with a mix of male and hermaphrodite inflorescences. These male-hermaphrodite mix trees can produce a few seeds [3].

Female trees are trees with mainly female inflorescences and produce seed [3].

Hermaphrodite trees are trees with mainly hermaphrodite inflorescences. They mainly produce seed but can also father some seeds as they produce pollen. Hermaphrodite trees may vary in their sex, becoming more female or male in the mast year [3].

4 Environment

Common ash grows throughout Europe but is absent from the driest Mediterranean areas as it does not tolerate extended summer drought, and from the northern boreal regions, because its seedlings are vulnerable to late spring frost [1, 2, 3]. It grows best on rich soils where soil pH exceeds 5.5, and soil controls its local distribution. Ash tolerates seasonal waterlogging, but not prolonged flooding [2]. It is a scattered tree species and rarely forms pure stands; it is more often found in small groups within mixed stands [2].

5 Threats

The biggest threat to common ash is currently a fungus called *Hymenoscyphus fraxineus* (T. Kowalski) Baral, Queloz & Hosoya (previously *Chalara fraxinea*). The disease was first discovered in Poland in 1992, and is now widespread throughout Europe with up to 80-90% of trees affected in many countries. The symptoms include

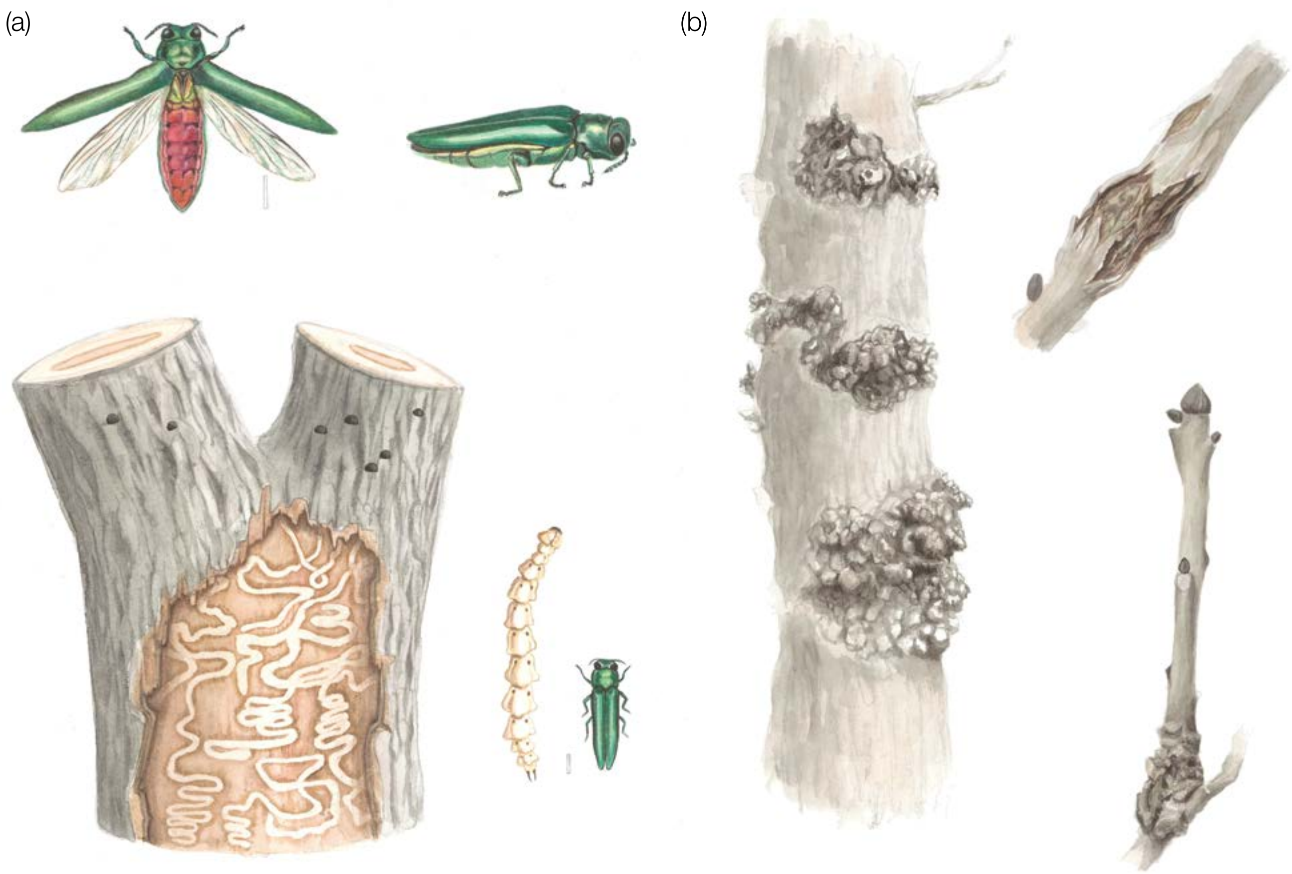

Figure 3: Emerald ash borer, an emerging threat to common ash (a) and ash canker (b).

severe defoliation, wilting, bark necrosis on stems and discolouration of the wood. Ash trees of all sizes (adult trees, saplings and seedlings) are affected. The disease has been observed to spread up to 20-30 km/year. In addition to spores, the disease can also spread via plant material. Other threats to ash health are ash cankers *Neonectria ditissima* (Tul. & C. Tul.) Samuels & Rossman and *Pseudomonas savastanoi* (Janse) Gardan, *et al*., *Phyllactinia fraxini* (DC.) Fuss*, Armillaria gallica* Marxm. & Romagn. and others [2, 4] (Figure 3b).

A potentially devastating threat is the emerald ash borer (*Agrilus planipennis* Fairmaire), a beetle native to Asia and eastern Russia (Figure 3a). While its adults graze on ash leaves, the larvae feed on the phloem, killing the tree. The borer was observed in western Russia and Sweden in 2007, and there is a strong concern that it might spread to all of Europe, devastating ash as it did in the US [2, 4].

6 Plot establishment and maintenance

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. Diameter at breast height (DBH) and social class can be used as a proxy 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 the coordinates of all trees taken. At the same time DBH can be measured and samples for DNA extraction taken.

Because common ash is most commonly a scattered tree species[1], a preliminary field study is needed; the size and shape of the genetic monitoring plot will need to be adapted to include 50 reproducing trees. Twenty-five of these should be functionally female and 25 functionally male. Hermaphrodite trees are often functionally female as they produce a fair amount of seed. As these hermaphrodite trees may vary in their sex, becoming more female or male in the mast year, the actual share of functionally female and male trees may change over the years.

Equipment needed:

- a device for distance measurement (a pair of range-finding binoculars is recommended)
- a compass
- paint and 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 Plot selection

To establish a monitoring plot for *F. excelsior*, ideally the initial work should be carried out in spring, when the trees are flowering. At this time, all ash trees in the stand should be mapped using a GPS device, and their sex recorded. In the summer, when trees are bearing fruit, the functional sex should be recorded for the hermaphrodite trees.

After the sex (and functional sex in the case of hermaphrodites) has been recorded, the GPS locations of all trees should be plotted as a point feature layer in GIS software. Fifty points representing trees, with a minimum distance of 30 m from each other, should be randomly selected keeping the ratio of 50% functionally male and 50% functionally female trees, including male, female and hermaphrodite trees. To account for GPS measuring errors we recommend looking for trees separated by more than 35 m (adjusting the minimum distance to 35 m). During plot installation, these pre-selected trees must be identified in the field and marked (Figure 4a).

[1] Common ash has a scattered distribution in the majority of its natural distribution range. In locations where it forms stands, the FGM plot must be established according to the guidelines for stand forming species, such as the European beech (*Fagus sylvatica* L.).

If two visits for recording tree sex are not possible, plot establishment should be carried out in the summer, recording and later randomly selecting 25 functionally male (trees not bearing fruit) and 25 functionally female (fruit-bearing) trees.

6.1.2 Plot installation in the field

Using the GPS, trees that were randomly selected in the office are located in the forest stand and marked. The minimum distance of 30 m between trees needs to be checked again.

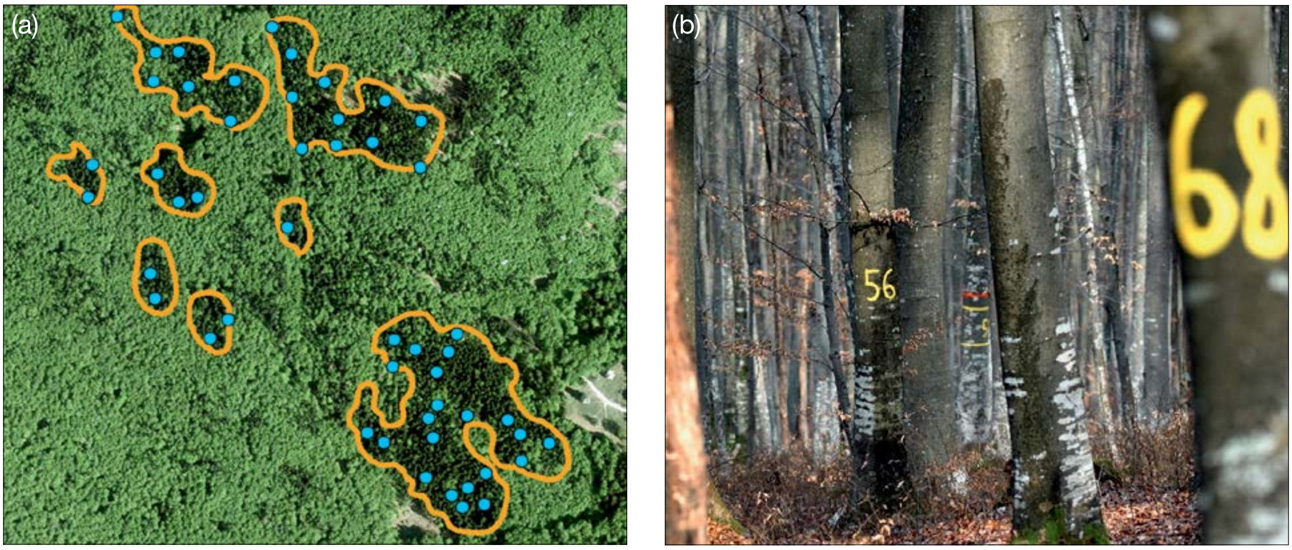

Figure 4: Plotted locations of randomly selected trees growing in multiple clusters (a); Each tree selected for genetic monitoring must be labelled with a corresponding number (image depicts the *Fagus sylvatica* FGM plot in Slovenia). To improve the visibility of selected trees from all directions, a band can also be painted around their trunks.

6.1.3 Labelling of trees

Each selected tree must be marked with a corresponding number (1 to 50) (Figure 4b), and preferably a band painted around the trunk to aid the visibility of the tree from all directions.

6.2 Establishment of natural regeneration subplots

The establishment of natural regeneration (NR) subplots should be carried out during germination two or more years after a strong or massive fructification event; the interval depends on the length of seed dormancy in a particular population.

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. 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. 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 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. If ash dieback is present in the stand, trees are monitored until they have reached stage 6, as set out in the background information Crown Dieback. Afterwards, they are replaced.

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 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 1: List of verifiers and background information with short descriptions and observation frequency to be recorded during fieldwork at the ash 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 2nd and 7th years after every assessed fructification event **	Counting of seedlings in the 2nd, 7th, 12th, and 17th years after every assessed fructification event **
Background information	DBH class distribution	/	Measurement every 10 years	Same as standard level
	Height class distribution	/	Measurement every 10 years	Same as standard level
	Sex ratio	/	Individual tree level observation, at the same time as the verifier Flowering	Individual tree level observation of the percentage of each inflorescence type, at the same time as the verifier Flowering
	Crown dieback	Individual tree level observation, every year	Same as basic level	Same as basic level
	Budburst	/	Individual tree level observation, 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.

** Ash has dormant seed; usually dormancy lasts for two winters. Therefore, natural regeneration abundance is first recorded two years after the major fructification event. If seeds are dormant for longer or shorter in the monitored ash stand, the observation years must adapt to the duration of the dormancy.

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 level

Verifier for mortality of adult trees. It 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.

7.1.1.2 Natural regeneration: Standard and advanced level

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 be recorded simultaneously with the background information 7.2.3 Sex ratio during flowering in March to 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 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 when flowering is in full progress.

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

Background information on flowering synchronisation can be estimated from the scores for flowering stage and the background information 7.2.3 Sex ratio.

Code	Flowering stage
1	Buds are closed, swelling of buds can be observed but stamens/pistils are not yet visible
2	Buds are open, stamens/pistils are visible but not yet shedding pollen/receptive
3	Inflorescences are fully open, stamens releasing pollen, pistils receptive

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

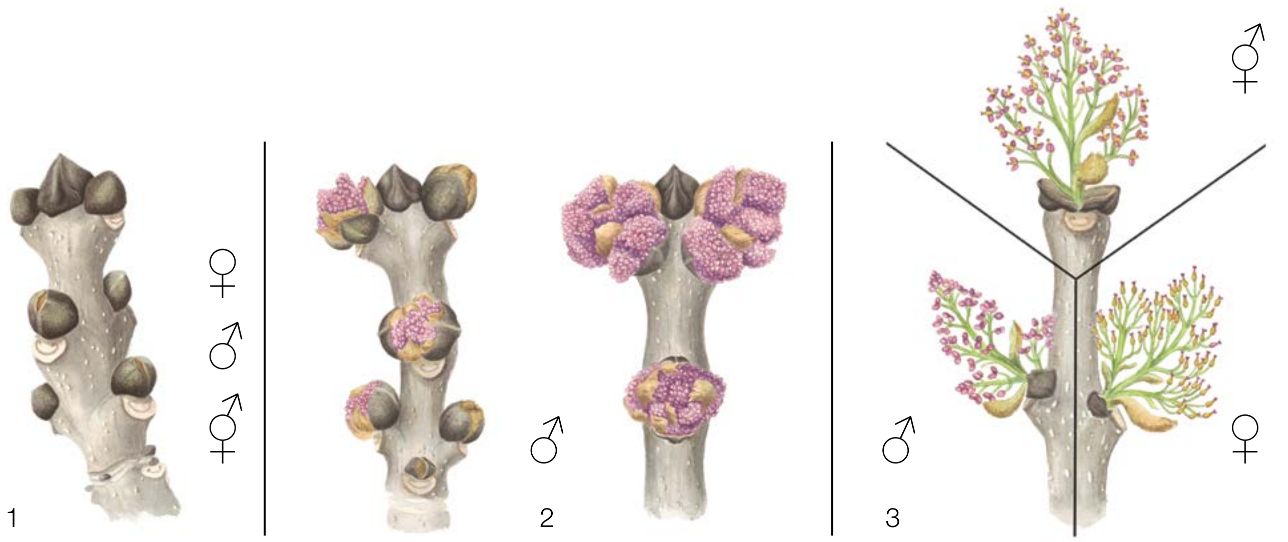

Figure 5: Picture guide for description of flowering stages for the advanced level verifier Flowering. For stage 3, the inflorescence growing from the terminal bud is only for illustrative purposes; in reality leaves develop from the terminal bud.

7.1.3 Fructification

This verifier describes the presence of fructification and its abundance. Data for this verifier should be collected during fructification, in August to October 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 fruit appearing on trees	0 – 10
2	Weak fructification: Some fruit appearing on trees	> 10 – 30
3	Moderate fructification: Moderate amount of fruit appearing on trees	> 30 – 60
4	Strong fructification: Abundant amount of fruit appearing on trees	> 60 – 90
5	Massive: Huge amount of fruit 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 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. 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 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

Indirectly, recording of this verifier provides information as to whether a tree is functionally female or male, and allows for observation of fluctuation of the functional sex through time.

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 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 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 fruits 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 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 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).

Code	Description: new natural 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 older than 1 year)
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 seedlings in the 2nd (in the autumn two years after the major fructification event; the year of the fructification event is regarded as year 0) and 7th years after the fructification event, as ash seeds usually remain dormant for two winters in the soil.

Counting of seedlings:
After the establishment of NR sublots, all ash seedlings present at each of the 20 NR sublots must be counted. Any older ash 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 – i.e., in the 8th year, five-year old saplings.

Number of seedlings counted on a subplot
X

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

The establishment of NR subplots and the beginning of observations must adapt to the actual duration of the seed dormancy in the monitored location.

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 of the 20 NR subplots in the 2nd autumn after every assessed fructification event (the year of the fructification event is regarded as year 0) and 7th, 12th, and 17th years after this fructification event, as ash seeds usually remain dormant for two winters in the soil.

If seeds are dormant for longer in the monitored ash stand, the observation years must adapt to the duration of the dormancy.

Table 2: Timeline of natural regeneration abundance (NR) assessment. In this example, the first assessed fructification event takes place in the 2nd year of the monitoring decade, and – considering ash seed dormancy of two winters – 20 NR subplots are established in the 4th year of the monitoring decade. The next assessment of fructification is carried out in the 8th year of the monitoring decade. Considering ash seed dormancy, 20 new NR subplots are established in the 10th year of the decade. 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	24	25
Fructification event		•			•	•		•		•		•		•			•	•		•		•		•	
NR assessment from the 1st assessed fructification event		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
NR subplots establishment				SA																					
NR abundance counting				SA					SA					A					A						
NR assessment from the 2nd assessed fructification event								0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
NR subplots establishment										SA															
NR abundance counting										SA					SA					A					A

Mortality/survival of natural regeneration is calculated from the numbers 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 level

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 a multi-trunk one 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 cases you would need to measure two perpendicular diameters and take the average
2) measuring the circumference of the tree and computing the diameter from that value (i.e. dividing by π, ~3.14) or using 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 level

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 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 Sex ratio

This background information describes the sex of individual ash trees. It can be recorded simultaneously with the verifier 7.1.3 Flowering during flowering in March to April in central Europe.

7.2.3.1 Standard level

At the standard level, this background information is recorded on the individual tree level on all 50 monitored trees at the same time as the verifier Flowering. For a graphical representation of male, female and hermaphrodite inflorescences, see Figure 6.

Code	Sex	Description
1	Male	More than half of inflorescences on the tree are male.
2	Female	More than half of inflorescences on the tree are female.
3	Hermaphrodite	More than half of inflorescences on the tree are hermaphrodite

7.2.3.2 Advanced level

At the advanced level, this background information is recorded on the individual tree level on all 50 monitored trees at the same time as the verifier Flowering. The percentage of male, female and hermaphrodite inflorescences is reported for each monitored tree with 10% accuracy. For a graphical representation of male, female and hermaphrodite inflorescences, see Figure 6.

Code	Sex
1	% male inflorescences
2	% female inflorescences
3	% hermaphrodite inflorescences

Figure 6: Picture guide for *F. excelsior* inflorescences for sex ratio determination.

Trees with inflorescences that are intermediate between female and hermaphrodite, with small anthers and which may or may not shed pollen are difficult to characterise. If more inflorescences are female, the tree may be characterised as female, if more are hermaphrodite, the tree may be characterised as hermaphrodite.

7.2.4 Crown dieback

This background information describes the crown condition due to ash dieback. The verifier is recorded every year by expert observation on all 50 monitored trees. Data for this verifier should be ideally collected during the period when the leaves are fully developed, e.g. in July in central Europe. For a graphical representation of stages of crown dieback, see Figure 7.

7.2.4.1 Basic, standard and advanced levels

Code	Description
1	healthy crown (0-10% defoliation)
2	dead branch tips visible on the crown’s edge, crown otherwise in good condition (11-30% defoliation)
3	dead branches visible on the crown’s edge, crown is thin enough that one can see through it (31-50% defoliation)
4	secondary crown is building at the trunk, thick branches without leaves visible, crown is very thin (51-80% defoliation)
5	only a small part of the crown remains (81-99% defoliation)
6	tree is dead (100% defoliation)

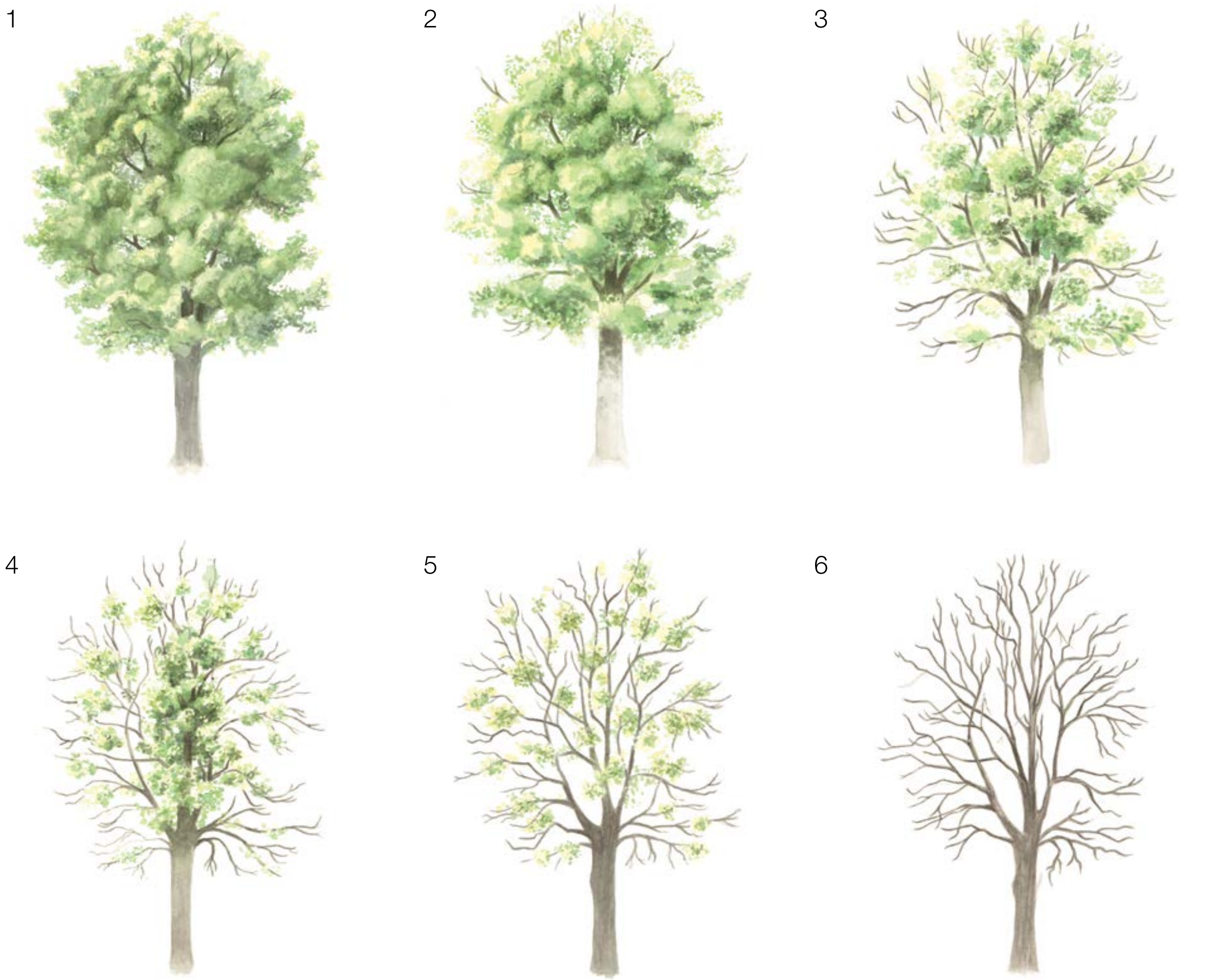

Figure 7: Picture guide for ash dieback estimation

7.2.5 Budburst

Budburst describes the process of budbursting (flushing). In ash, budbursting starts after flowering. Recording is only carried out at the standard and advanced levels. Data for this background information should be collected in April in central Europe; several visits are needed and recording stops when all monitored trees have reached fully developed leaves. Budbursting is earlier when preceded by a warm winter.

7.2.5.1 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 3) and the end of budbursting (stage 5). The observations cease 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 proportion of the crown budbursting. For a graphical representation of budbursting stages, see Figure 8.

Code	Stage of budbursting
1	Dormant buds
2	Buds are swelling but are still closed
3	Buds are bursting
4	Buds are elongating
5	Leaves are separated and start growing vertically

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

7.2.5.2 Advanced level

At advanced level, budburst is recorded on an individual tree level on all 50 monitored trees every year in the same way as at the standard level. For details see 7.2.5.1 Standard level.

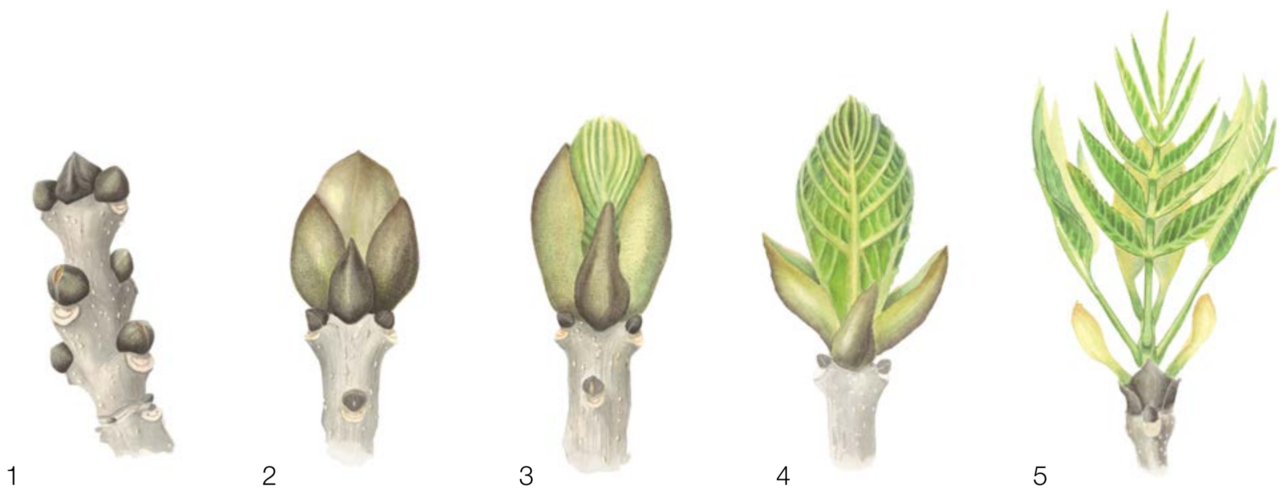

Figure 8: Picture guide for description of budburst (flushing) for the standard and advanced level background information Budburst.

7.2.6 Senescence

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

7.2.6.1 Standard level

At the standard level, senescence is recorded on an individual tree level on all 50 monitored trees every five years. We are looking for stage 3, when leaves are yellow and do not photosynthesise anymore. Observations stop when all the trees have reached stage 3. Usually, two (2) visits to the plot will be needed. For each tree, three estimates are given: stage of leaf colouring, proportion of the crown senescing and proportion of the leaves being shed.

Code	Stage of leaf colouring
1	Leaves are fully green
2	Leaves are green with yellow spots
3	Leaves are fully yellow
4	Leaves are brown

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

Code	Proportion of leaves that have been shed due to senescence (%)
1	> 0 – 33
2	> 33 – 66
3	> 66 – 99
4	100

7.2.6.2 Advanced level

At the advanced level, senescence is recorded on an individual tree level on all 50 monitored trees every year in the same way as at the standard level. For details see 7.2.6.1 Standard level.

7.2.7 Flowering synchronisation

7.2.7.1 Advanced level

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 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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