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Guidelines for genetic monitoring of

Pedunculate oak
(*Quercus robur* L.)

and

Sessile oak
(*Quercus petraea* (Matt.) Liebl.)

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Guidelines for genetic monitoring of

9.2.7 Pedunculate oak (*Quercus robur* L.) *and* Sessile oak (*Quercus petraea* (Matt.) Liebl.)

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1 Executive summary

Among the 13 European white oak species, pedunculate (*Quercus robur* L.) and sessile (*Q. petraea* (Matt.) Liebl.) oak are the most important deciduous forest tree species in Europe, both economically and ecologically. Both oak species are widely distributed in Europe; from northern Spain to southern Scandinavia and from Ireland to Eastern Europe. Moreover, both are closely related; they can mix, compete and naturally hybridise with one another and with other oak [2, 3, 5, 8].

Oak are among the most diverse species of forest trees. High levels of diversity are most likely due to the maintenance of large population sizes, overlapping of ecological niches, long-distance gene flow and interfertility. The human impact on oak populations is very large, and most oak forests are managed, primeval forests like Bialowieza in Poland and Belarus being very rare. Genetic resources of oak are endangered not only due to the loss of natural ecosystems and limitation of seed sources, but also by the impact of several decades of air pollution and by long-term climate changes [3].

These guidelines briefly describe pedunculate and sessile oak, their reproduction, ecology, importance, distribution and threats. They provide guidance on establishing a genetic monitoring of *Quercur robur/petraea* complex and on recording all field level verifiers.

2 Species description

Pedunculate oak (*Quercus robur*) and sessile oak (*Q. petraea*) are large deciduous trees that reach 30 – 40 m in height and live up to 800 years or more. These two tree species, as well as other oak, are very variable morphologically, and can naturally hybridise, generating individuals showing intermediate traits or the prevalence of one, so that it can be difficult to characterise them unequivocally by observations alone [1, 2, 3, 5].

Both the pedunculate and sessile oak have an extended and mostly overlapping distribution throughout most of Europe. Their natural range extends from Ireland and northwestern parts of the Pyrenean peninsula in the west, to Eastern Europe in the east, southern parts of Scandinavia in the north, while their southerly range limits are more difficult to define, as these oak can mix, compete and naturally hybridize with other Mediterranean oak, such as *Quercus pubescens* Willd. and *Quercus frainetto* Ten., even if at relatively low rates [3, 5]. In the east the pedunculate oak has a more extended distribution, reaching the Ural Mountains, while the sessile oak's extends to Ukraine.

The main differences between pedunculated and sessile oak are the characteristics of the leaves, fruits and trunks.

The main trunk of *Q. robur* tends to disappear in the crown, developing irregular boughs with twisting branches (Figure 1), while *Q. petraea* usually develops a main stem with boughs gradually decreasing in size (Figure 2) [1, 2, 3, 5]. The bark of both species is grey, fissured, forming rectangular elongate blocks, which are thicker in *Q. robur*, while those of *Q. petraea* often tend to exfoliate.

The leaves are simple, obovate-oblong and deeply and irregularly lobed, with a short stalk (2-7 mm) in *Q. robur* and a long stalk (13-25 mm) in *Q. petraea* (Figure 3) [1, 2, 3, 5].

The fruits are acorns, which are often in pairs and sit in scaly cups on the ends of long stalks in *Q. robur* and on short or absent stalks in *Q. petraea*. The acorns are very variable in size and shape, but those of *Q. robur* are usually smaller and rounded with olive-green longitudinal stripes visible when fresh [1, 2, 3, 5].

It can be sometimes difficult to characterize them only by visual observations, and molecular methods are the most reliable tool for species differentiation. When in the field, leaves and acorn characteristics are the key features to determine the species and define the level of hybridisation, between sessile and pedunculate oak in mixed populations. The main criterion for the taxonomic determination of sessile and pedunculate oak hybrids is the intermediate value of several morphological signs considering typical values for each species. The best basis

Figure 1: Pedunculate oak (*Quercus robur*) habitus in summer and winter

Figure 2: Sessile oak (*Quercus petraea*) habitus in summer and winter

for differentiation is provided by length of leaf stalk (sessile oak – long, pedunculate oak – short), length of acorn stalk (sessile oak – short, pedunculate oak – long), leaf indentations (sessile oak – not so deep, pedunculate oak – deep), leaf veins in the indentations (sessile oak – not present, pedunculate oak – very common), the shape of leaf base (sessile oak – clinal or unpronounced lobes, pedunculate oak – very pronounced lobes), leaf hairs (sessile oak – flat-lying, stellate hairs over leaf underside, pedunculate oak – no hairs) [1, 2, 3, 5].

Figure 3: Leaves and fruit of pedunculate oak (*Quercus robur*) (left) and sessile oak (*Q. petraea*) (right)

3 Reproduction

These oak are monoecious and wind-pollinated, with distinct male and female flowers borne on two types of inflorescences [1, 2, 3, 5, 6].

Male flowers are grouped in catkins, about 5 cm long; they develop in the axils of either the inner bud scales or the first leaves. Both species bloom in late spring (the end of April and in May), together with leaf expansion (*Q. robur* two weeks before *Q. petraea*). For a given tree, if weather conditions are suitable, catkin growth is achieved in 1-2 weeks after bud opening, and pollination is completed in 2-4 days [1, 2, 3, 5, 6].

Female flowers appear at terminal shoots just after the first leaves (and male catkins) have flushed. They are globular and only 1 mm in size, thus being very inconspicuous and difficult to observe. When receptive, they become sticky and reddish. On *Q. robur* they appear individually or in small groups on long stems, while on *Q. petraea* they are sedentary and grow in groups of 2 – 5 [1, 2, 3, 5, 6].

Allogamy is supported by several mechanisms, like different male and female flowering times on the same tree, physiological advantages of foreign pollen, the same trees in the stand do not flower and fructify every year, etc. [3,6].

After fertilisation, the acorns mature within approximately 3 months, then fall off the tree. Acorns of *Q. robur* mature at the end of September or beginning of October, earlier than the acorns of *Q. petraea*, which mature in October [1, 2, 3, 5]. Trees usually start fructifying at the age of between 40 and 100 years, in coppice stands at 20. Mast years usually occur every 5 to 7 years and vary according to individual tree, population, region, year and tree density (a low density favours earlier reproductive maturity) [3].

The reproduction of oak is done mainly with seed. Mammals and birds are important for seed dispersal, especially the Eurasian jay (*Garrulus glandarius* L.), which can be considered the primary propagator, since it can spread seeds up to 5 km in distance. The capacity for stump sprouting may be present in juveniles and, although decreasing with the age of the trunk, may enable oak to maintain their populations even in the absence of acorn production. In contrast to pollen and acorn dispersal, vegetative propagation is not an important component of gene flow. It can, however, participate in the maintenance of genetic variability within a population [2, 3, 4, 5, 6].

4 Environment

Q. robur and *Q. petraea* co-occur at many sites as a main component of temperate deciduous mixed forests, and they share several common characteristics. These oak are vigorous trees with large ecological amplitude, although they prefer fertile and moist soils, and are able to dominate forests in number and size at low-mid elevations. Both are able to behave as pioneer trees, are rarely affected by late frost due to late budbursting (flushing), and have a good re-sprouting attitude, so they coppice and pollard easily. Their deep and penetrating taproots (more developed in *Q. petraea*) give them structural stability against windthrow and allow them to withstand moderate droughts by accessing deeper water. However, in conditions far from their optimum, they show ecological differences. The tendency is for *Q. robur* to grow on heavier soils in more continental climates, in wet lowlands and damp areas by streams and rivers, tolerating periodic flooding. *Q. petraea* is more tolerant of drought and poor soil than pedunculate oak, but more sensitive to heavy soil conditions. It prefers to grow in more Atlantic climates on light and well-drained, often rocky, soils, generally occurring on slopes and hill tops, and preferring a more acidic soil. They are both light-demanding trees (*Q. robur* more so than *Q. petraea*), and their canopies permit a good deal of light to pass through to the undergrowth, promoting the regeneration of many tree species and enriching forest diversity. These oak rarely form pure forests under natural conditions. On plains, plateaus and hills, pedunculate oak is a pioneer species and sessile oak a late successional species. Sessile oak can reach the climax stage if summers are dry. In valleys and floodplains pedunculate oak is a late successional species which reaches climax with sycamore, plane, maple, ash and elm [2, 3, 5].

5 Threats

Oak populations are at risk because of climate change and fragmentation of their habitats (mostly *Q. robur* in lowland), changes in groundwater regime and over-exploitation of mature trees [2].

The big threat to the genetic diversity of the oak is the introduction of exotic genotypes through plantations. Populations that occupy more extreme habitats are at a particularly high risk of disappearing, because the number of individuals is low, habitats are unstable and human impact is often considerable [3].

Due to the unbalanced ratio of development phases, over-abundance of game or changes in groundwater regime natural regeneration can be limited. Often, seedlings die within a couple of years after germination [1, 2].

Pests and pathogens also represent a serious threat. Oak mildew (*Erysiphe alphitoides* (Griffon & Maubl.) U. Braun & S. Takam) is reported to be the most common oak pathogen. Acute Oak Decline is a new syndrome principally affecting pedunculate and sessile oak, characterised by a decrease in the density of the crown, the appearance of dark oozing wounds ('bleeds') on the trunk, and in most cases the presence of the jewel beetle *Agrilus biguttatus* Fabricius [3].

Defoliation of the first flush of leaves is common and caused by caterpillars of several butterfly species, e.g. *Tortrix viridana* L., *Lymantria dispar* L., *Operophtera brumata* L. *and Thaumetopoea processionea* L.. Knopper gall wasps (*Andricus quercuscalicis* Burgsdorf) can also cause some damage to acorn crops [3].

6 Plot establishment and maintenance

Since oak forms almost pure or mixed stands with many lowland forest tree species, establishment of a regular forest genetic monitoring (FGM) plot, as for other stand-forming species, should be followed with 50 reproducing trees. These are dominant or subdominant trees that are phenotypically appropriate and are at least 30 m apart and will contribute to new generations. If a tree is flowering, it is regarded as a reproducing tree.

The best time for FGM plot establishment and tree selection is in the spring, when reproducing trees are flowering and leaves and acorns for species (hybrid) determination can be collected from the ground. In cases when the plot cannot be established in the flowering season, DBH and social class can be used as a proxy to identify a reproducing tree, 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.

Due to natural crossbreeding between oak, it is advisable to perform morphometric analyses of fallen leaves and acorns to determine the species and population taxonomy of the forest stand before establishing a genetic monitoring plot. The main criteria for the taxonomic determination of oak hybrids are described in species description.

Equipment needed:

- a device for distance measurement (a pair of range-finding binoculars is recommended),
- a compass,
- a 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 Selection of the centre of the plot

The general procedure for random plot site selection consists of the following steps (Figure 4a):

- 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 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 does not meet this demand, a new point must be selected following the protocol described above.

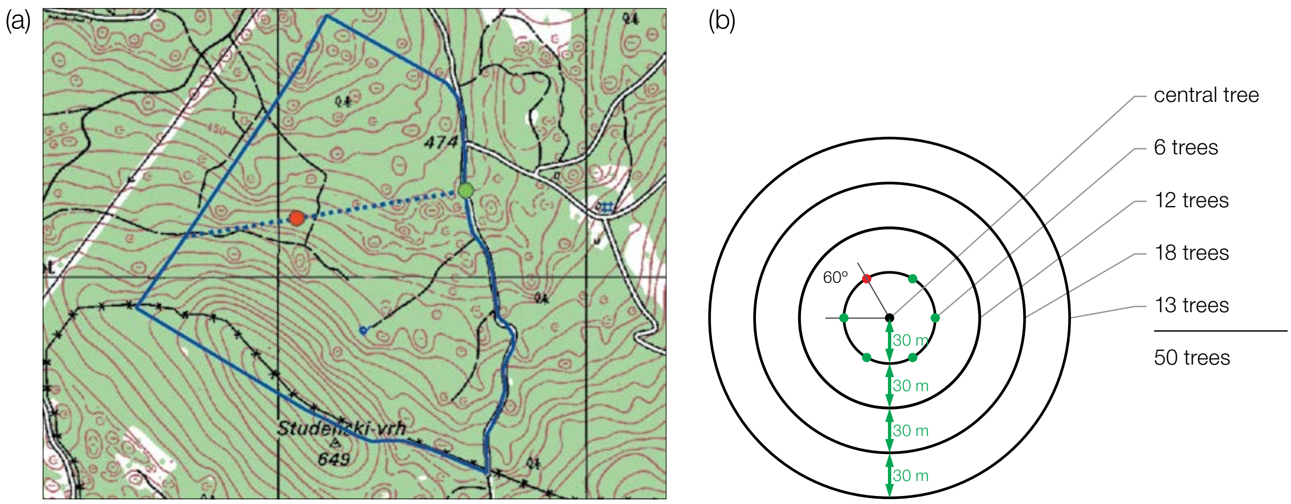

Figure 4: Random selection of the centre of the FGM plot (a); selection of trees in concentric circles around previously selected central tree with an increasing radius of 30 m (b).

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

The selected point's coordinates are to 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 4b). 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 4b), additional trees are selected in the outermost circle.

Table 1: Random azimuths to 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

Each selected tree must be marked with a corresponding number and preferably 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 5a). 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 5b).

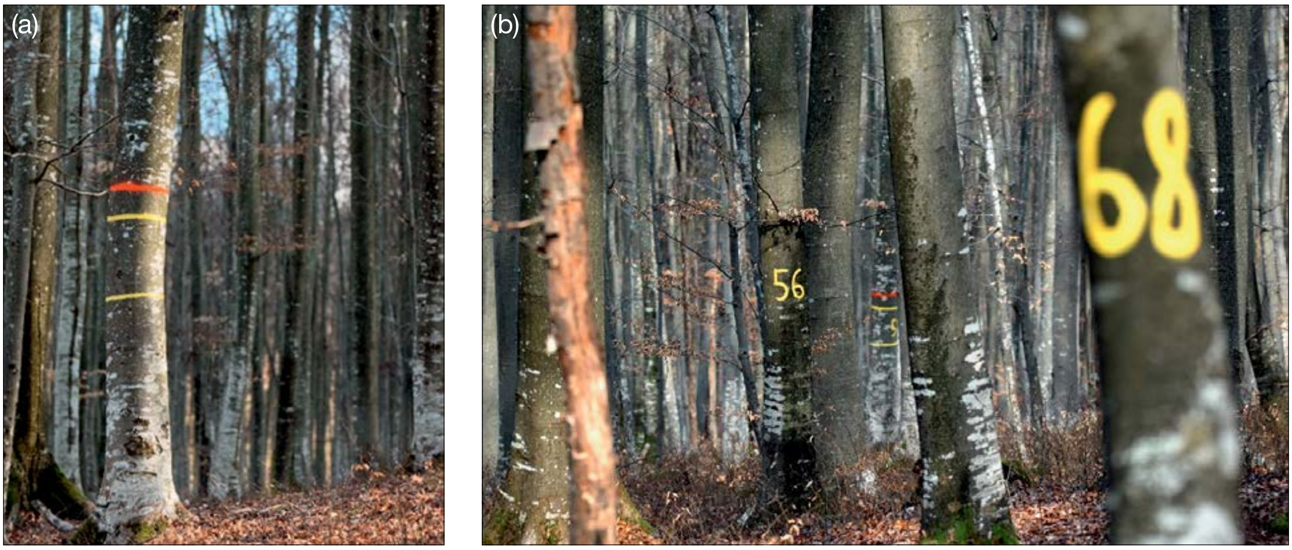

Figure 5: a) The central tree on the genetic monitoring plot is marked with multiple bands to differentiate it from other trees; b) numbers are painted on selected trees so that they point away from the central tree. Both images depict a *Fagus sylvatica* genetic monitoring plot.

6.2 Establishment of natural regeneration subplots

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

Natural regeneration centres from the last major fructification 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 the logged NR centres, 20 should be chosen randomly for plot installation. If 20 or fewer NR centres are present, all should be used.

Inside each selected NR 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 NR subplot markings must be checked periodically (every 2 years) and renewed if needed.

6.3.2 Replacement of trees

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

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 the oak 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 the 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 1st and 6th years after every assessed fructification event	Counting of seedlings 1st, 6th, 11th, 16th 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
	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

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 marked trees remaining alive every 10 years and after every extreme weather event/disturbance. Mortality is the difference between the initial number of marked trees and the trees remaining alive of the original 50.

7.1.1.2 Natural regeneration: Standard and advanced levels

Mortality is the difference between the initial number of plants and the plants remaining alive at the time of the next counting. For each assessment of abundance natural regeneration plants are first counted in the year of germination and then 5 years later at the standard level, while at the advanced level counting is also performed 10 and 15 years after germination. NR abundance assessment 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 in April to May in central Europe. Flowering is earlier when preceded by a warm winter.

Male flowers (Figure 7): The criterion for determining the beginning of flowering is defined by the development of catkins. Male flowers (catkins) begin to develop immediately after first leaves appear, the release of pollen begins when catkins lengthen and thicken. The end of flowering of male flowers appears when there are no pollen active male flowers in the crown. The colour of catkins turns dark brown and the consistency is like a cobweb.

Female flowers (Figure 6): Female flowers in oak are very small and hardly visible; therefore all assessments of flowering are focused only on male flowers. Consequently, and in contrast to most other species, the background information Flowering Synchronisation is not monitored for pedunculate and sessile oak.

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 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 of the flowering stage	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.

Two scores are provided for each tree: male flowering stage and the proportion of the crown flowering. Because female flowers in pedunculate and sessile oak are very small and inconspicuous, the assessment of the female flowering stage cannot be done reliably in practice. The proportion of the crown flowering refers to the total amount of male flowers on the tree. For a graphical representation of female flowers and male flowering stages see Figures 6 and 7.

Code	Male flowering stage
1	Elongated peduncle – closed flowers (green)
2	Anthers releasing pollen (yellow)
3	Empty anthers (pollen released) (brown)

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

(a) (b)

Figure 6: Female flowers of the pedunculate oak *(Quercus robur)* (a), and sessile oak *(Q. petraea)* (b). Female flowering is not assessed, as female flowers of both species are too inconspicuous to observe reliably in the field.

1 2 3

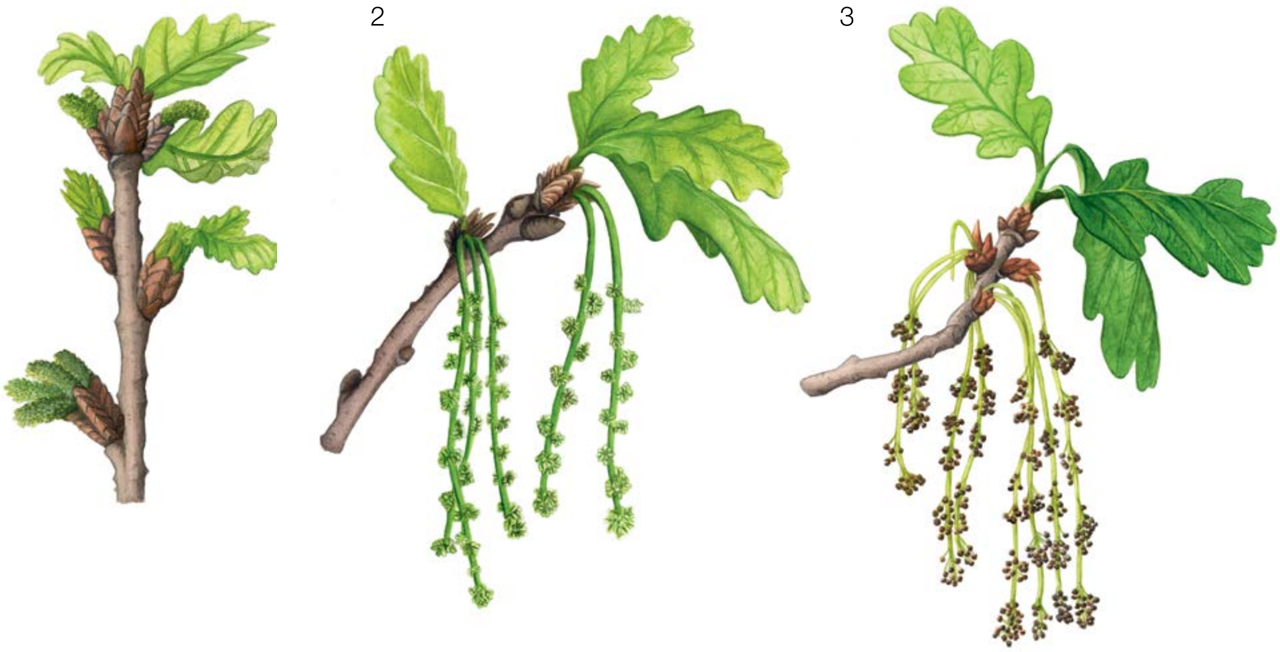

Figure 7: Picture guide for description of male flowering stages for the advanced level verifier Flowering, for pedunculate and sessile oak.

7.1.3 Fructification

This verifier describes the presence of fructification and its abundance. Data for this verifier should be collected during fructification, in September to October in central Europe. Acorns of *Q. robur* mature at the end of September or beginning of October, earlier than the acorns of *Q. petraea*, which mature in October.

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 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 the fruit, i.e. acorns, starts 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, 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

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 fruit, i.e. acorns, starts falling. One score is provided for each tree. Simultaneously, acorns are 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).

This verifier is recorded by counting fruit using binoculars. The average of three rounds of counting is reported. Each round of counting consists of the number of fruits 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 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 (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 NR’ (seedlings that germinated the same year as the observation is carried out, Figure 8) and one for the ‘established NR’ (saplings older than ‘new NR’).

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

Figure 8: A one-year old seedling.

7.1.4.2 Standard level

This verifier is recorded by counting seedlings in 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.

Oak seeds are not dormant and can already sprout in the year following fructification. Because major fructification of pedunculate and sessile oak occurs approximately every 5 to 7 years, the next round of monitoring of natural regeneration abundance follows after the next major fructification event (approximately 5 to 7 years after the establishment of the previous subplots).

Counting of seedlings:

After the establishment of NR subplots all oak seedlings present at each of the 20 NR sublots must be counted. Any older oak saplings that are present on the NR subplot must not be included. During the next round of counting, only saplings of the appropriate age must be counted – 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

The verifier is recorded by counting seedlings at each of the 20 NR subplots in the 1st autumn after every assessed fructification event (the year of the fructification event is regarded as year 0) and 6th, 11th, 16th autumns after the fructification event. Only plants of the appropriate age must be counted – in the 6th year, 5-year old saplings, in the 11th year, 10-year old saplings etc.

Table 3: Timeline of natural regeneration abundance (NR) assessment. In this example, the first 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. 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 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, measure all of them and record the average (but try to avoid trees with many small trunks). Note that the tree is multi-trunk in the notes and include the number of trunks measured. If the tree is leaning, measure DBH perpendicular to the tree trunk. DBH can be measured in two ways:

1) using a calliper, in which case you 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 metres to one decimal place. If the crown is damaged, this must be recorded as well as the stipulated reason in notes.

7.2.3 Budburst

Budburst describes the process of budbursting (flushing). Recording of this parameter is only carried out at the standard and advanced levels. In pedunculated and sessile oak, budbursting starts together with flowering (*Q. robur* two weeks before *Q. petraea).* Data for this verifier should be collected in April and May in central Europe until all monitored trees have reached fully developed leaves. Budbursting is earlier when preceded by a warm winter.

7.2.3.1 Standard level

At standard level, budburst is recorded on an individual tree level on all 50 monitored trees every 5 years. 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, 6 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 9.

Code	Stage of budbursting
1	Buds completely closed (no green is visible)
2	Buds begin to burst (first green is visible)
3	Folded and hairy leaves begin to appear; individually visible folded and hairy leaves
4	Leaves fully unfolded, smooth and bright

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 the 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 7.2.3.1 Standard level.

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

7.2.4 Senescence

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

7.2.4.1 Standard level

At standard level, senescence is recorded on an individual tree level on all 50 monitored trees every 5 years. 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, two estimates are given: stage of senescence and proportion of the crown senescing.

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

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

7.2.4.2 Advanced level

Senescence is recorded on an individual tree level on all 50 monitored trees every year in the same way as at the standard level. For details see 7.2.4.1 Standard 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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