**D2.1 – Common protocols and reference standards for selected traits and species**

**Description:** Common protocols and reference standards for selected traits and species: Technical cards are drawn up holding common protocols and reference standards at a species or at a trait level, as appropriate, and will be made publicly available to the scientific community.

Due date of deliverable: M42  
Actual submission date: M42  
Organisation name of lead contractor for this deliverable: vTI (now THÜNEN)  
WP2 Leader: Bart de Cuyper (VLAGEW)  
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<table>
<thead>
<tr>
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<th></th>
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</thead>
<tbody>
<tr>
<td>PU Public (must be available on the website)</td>
<td>X</td>
</tr>
<tr>
<td>PP Restricted to other programme participants (including the Commission Services)</td>
<td></td>
</tr>
<tr>
<td>RE Restricted to a group specified by the consortium (including the Commission Services)</td>
<td></td>
</tr>
<tr>
<td>CO Confidential, only for members of the consortium (including the Commission Services)</td>
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5. Reference genotypes
1. Summary

Systematic provenance and progeny trials have been established for more than 100 years. Many measurements and assessments were carried out within these trials. Standards were defined for the traditional measurements like height and diameter (dbh). Numerous scoring schemes were developed for other traits. This makes it difficult or not feasible to compare results between institutions, but also between test series and single trials. Within the FP7 project TREES4FUTURE, this topic is addressed in “Work package 2 – Creating common standards and protocols”.

The deliverable of “Task 2.1 – Development of common protocols and selection of reference genotypes” presents species specific, and non-species specific common protocols for five broadleaves (species or species groups), and five conifers (species and species groups), respectively. The status and development of the assessment of wood characteristics and drought are given. Furthermore, the deliverable presents a concept and a selection of reference genotypes.

Key words: assessment, phenology, stem form, disease, wood quality, drought, reference genotype
2. Context

Many traits are of interest to forest tree breeders to distinguish genetic units, and to describe genetic variation and the evaluation of genotypes along their breeding programs. A lot of scoring systems have been developed by all tree breeders. This makes results between institutions but also between test series and single trials difficult or unfeasible to compare. Common protocols were used for a limited number of trial series. However, this is mostly infrequent.

Criteria for common assessments methods are: acceptable, simple, easy to handle (fast), low-cost, defined for a specific part in the life cycle.

The starting point for Task 2.1 was an inventory of existing national assessment schemes. The inventory could be assembled from the results of the FP6 project TREEBREEDEX (PÂQUES 2009, DUCCI et al. 2012). The inventory was essential because the tree species beech and oaks were not included in TREEBREEDEX. Further protocols drawn up from the literature were added. During the workshops a selection was taken and common protocols identified.

The task involves a large number of participants, each with their own species of interest, leading to a high number of species x traits combinations. Moreover, common protocols are usually not transferable from one species to another. Not all species x traits combinations can be tackled within the time span of the project, so priorities will have to be set regarding the species as well as regarding the traits to be assessed.

A selection was made of six target species (beech, ash, wild cherry, Norway spruce, Douglas fir, Scots pine) and four species groups (oaks, poplars, Mediterranean pines, larches) of high interest from a European point of view (Chapter 3).

The number of common protocols varied from one each of the beech and oak species to seven for the species group poplars. Furthermore common protocols were identified for broadleaves and conifers. For each species/species group a core group was created and a responsible core group leader determined.

Specific input was provided at the trait level for the assessment of wood characteristics and drought based on the results of questionnaires (Chapter 4).

Furthermore, the concept of reference genotypes and a selection of extreme reference genotypes with low genotype-environmental-interaction are presented (Chapter 5).

References


DUCCI F, DE CUYPER B, PÂQUES L, PROIETTI R, WOLF H (comp.), 2012: Reference protocols for assessment of trait and reference genotypes to be used as standards in international research projects. CRA SEL, Arezzo, Italy: 82 pp.
3. Species oriented common protocols

3.1 Broadleaves

3.11 Beech specific protocols

Core group: **Mirko Liesebach** (vTI now THÜNEN),
Sven M.G. de Vries (ALTERRA),
Marek Rzonca (IBL),
Ecaterina Nicoleta Chesnoiu (ICAS),
Ricardo Alia (INIA),
Alexis Ducousso (INRA)

### 3.11.1 Flushing (bud break)

**Definition**

A specific trait of beech (*Fagus sylvatica* L.) is **flushing (bud break)**, which is under strong genetic control. Results from provenance trials show large differences in leaves’ flushing time in spring. Generally, provenances from the eastern and south-eastern part of the distribution area, as well as provenances from high elevations, require a smaller heat sum for flushing and thus flush earlier. Provenances from the western part of the distribution and from low elevations where late frosts occur require a higher heat sum and flush late. Depending on the temperature development during spring, the time differences between the earliest and the latest individuals to flush in a set of provenances in a trial can be 4 to 6 weeks. Between the different years and over a number of sites, certain stability was found in the ranking of flushing time in the provenances analysed.

The phenology of flushing is important for damages by late frost. In the context of climate change that becomes more and more important. Damages by late frost can result in yield losses and poor stem forms. Therefore, flushing is a trait which has an indirect economic impact, apart from its adaptive value.

**Description of the assessment in general**

flushing will be recorded for each tree separately. Observations on beech show that there is no rule whether the terminal bud or other buds starts flushing.

Experience shows that in younger plants a stage is reached when approximately 3 buds show the specific characteristics. Flushing can easily be recorded at younger plants up to a height of 2 m (average height of a trial series)

For older and large plants it is almost impossible or at least not easy to record single buds, i.e., when the average height of a trial site is above 2 m. In this case a stage is reached when 50% of the crown shows the characteristics of a specific stage. This is according to the BBCH recommendations.
Depending on the scientific question and the financial resources, records will be taken
(1) once, all trees at a time (each stage) or
(2) periodically
(2.1) each stage, all trees at each assessment,
(2.2) one specific stage, only trees recently flushed at each assessment.

When the assessment should only be carried out once, the timing is important. The good
variation between the genetic units is around a mean value of the scoring schema for a site.

The assessment has to be carried out uninterrupted and should not last more than one day.
A replication should be assessed without a break.

The assessment of flushing should first be done at the beginning of the second vegetation
period in the field.

For older plants, the assessment is only to get an impression whether a genetic unit is
flushing early or late.

**Scoring schema**

a) For trees up to a mean height of 2 m of a trial series a 5-step-scale is recommended:

<table>
<thead>
<tr>
<th>Scale values</th>
<th>Text</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>dormant winter bud</td>
</tr>
<tr>
<td>2</td>
<td>buds expanding</td>
</tr>
<tr>
<td>3</td>
<td>bud-burst (first green is visible)</td>
</tr>
<tr>
<td>4</td>
<td>leaves are flushing</td>
</tr>
<tr>
<td>5</td>
<td>leaves are fully expanded</td>
</tr>
</tbody>
</table>

Stage 1
D2.1 – Common protocols and reference standards for selected traits and species

Stage 2

Stage 3

Stage 4
Stage 5

Photos: ALTERRA (left), M. LISEBACH (right)

b) For trees above a mean height of 2 m, a 3-step-scale is recommended:

<table>
<thead>
<tr>
<th>Scale values</th>
<th>Text</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (1)</td>
<td>dormant winter bud (late flushing)</td>
</tr>
<tr>
<td>2 (3)</td>
<td>bud-burst (first green is visible)  (intermediate)</td>
</tr>
<tr>
<td>3 (5)</td>
<td>leaves are fully expanded (early flushing)</td>
</tr>
</tbody>
</table>

(numbers in brackets correspond to the scale mentioned under 3.1)

Photos: ALTERRA

**Pros and cons of the described protocols**

a) Pros
There is good experience in using the protocols.
Genetic units can be distinguished nicely coming at the right time for the assessment.
Several assessment schemas were used in the past. The results of existing assessments can only be converted backwards with losses in a few steps (see Table 3.11.1) not vice versa. The data can be analysed as a quantitative trait by adjusting a Weibull function and can be easily compared among sites and recording dates.

Table 3.11.1: Assessment schemas used for scoring flushing at beech trials

<table>
<thead>
<tr>
<th>3-step</th>
<th>5-step</th>
<th>6-step</th>
<th>7 step</th>
</tr>
</thead>
<tbody>
<tr>
<td>1= late flushing</td>
<td>1= dormant winter bud</td>
<td>1= dormant winter bud</td>
<td>1= dormant winter bud</td>
</tr>
<tr>
<td>2= intermediate</td>
<td>2= buds expanding</td>
<td>2= buds swollen</td>
<td>2= buds swollen</td>
</tr>
<tr>
<td>3= early flushing</td>
<td>3= bud-burst (first green is visible)</td>
<td>3= first green is visible</td>
<td>3= first green is visible</td>
</tr>
<tr>
<td>4= leaves are flushing</td>
<td>4= leaves begin to appear, individually visible folded</td>
<td>4= leaves begin to appear, individually visible folded</td>
<td>5= individually visible folded</td>
</tr>
<tr>
<td>5= leaves are fully expanded</td>
<td>5= leaves unfolded, fan-shaped</td>
<td>6= leaves unfolded, fan-shaped</td>
<td>6= leaves unfolded, bright</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6= leaves unfolded, bright</td>
<td>7= leaves unfolded, bright</td>
</tr>
</tbody>
</table>

b) Cons

It is not always easy to catch the right time to have a good differentiation between the genetic units in flushing.

- Difficulties in choosing proper term of assessment,
- possible subjective influence,
- requires trained staff.

References


3.12 Oak (Quercus spp.) specific protocols

Core group:
Marek Rzońca (IBL),
Sven M.G. de Vries (ALTERRA),
Ecaterina Nicoleta Chesnoiu (ICAS),
Alexis Ducousso (INRA),
Mirko Liesebach (vTI now THÜNEN)

3.121 Flushing (bud break)

Definition
Tree phenology changes seasonally due to both climate and endogenous rhythms. A specific trait for oak species is **flushing**, (bud break). Bud break is the first visible step in the annual cycle of trees growth. In oaks species, early wood, including large vessels and a part of the annual radial stem growth is achieved before leaf expansion in spring. Oaks have two types of flushing, early and late. Long term observations show about 2-3 week differences between these types. In the southern range (northern Italy) flushing starts on about 20 April, in the northern range (southern Scandinavia) on 20 May (for Quercus petraea, Q. robur). Spring flushing dynamics and terms of dormancy are largely genetically determined. The variability of flushing is important for damages by late frost, causes in yield losses and poor stem forms. Consequently, flushing is a specific visible trait, which affects the oaks’ adaptive potential and economic value.

Description of the assessment in general
The assessment of oak flushing is similar to beech. Bud break should be assessed separately for each tree in the field.

Due to size of observed material (height of tree) flushing can be more easily recorded for younger tree, especially those up to 2 m of height. Taller trees should be assessed with another scale. In this case is not possible to observe single buds. The BBCH recommendation states that when 50 % of the crown achieves the specific characteristics, the stage is reached.

The issue of the date of assessment, it should be carried out in several ways:
- One time (all trees in one date)
- Periodically
  - In each stage (all trees at severals date and each assessment)
  - In only one specific stage (only trees recently flushed at each assessment)

When the assessment is carried out, the most important factors is to check for a good time for observation. If the assessment starts too early, buds are not variably developed and cannot be observed at all stages of the scale. The same problem appears when assessment starts too late. The good variation among trees is about a mean value of scoring schema for an each field trial.
**Scoring scheme**

a) For trees up to a mean height of 2 m, a 5-step-scale is recommended:

<table>
<thead>
<tr>
<th>Scale values</th>
<th>Text</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>dormant winter bud</td>
</tr>
<tr>
<td>2</td>
<td>buds expanding</td>
</tr>
<tr>
<td>3</td>
<td>bud-burst (first green is visible)</td>
</tr>
<tr>
<td>4</td>
<td>leaves are flushing</td>
</tr>
<tr>
<td>5</td>
<td>leaves are fully expanded</td>
</tr>
</tbody>
</table>

Photos: MLUV

b) For trees above a mean height of 2 m, a 3-steps-scale is recommended:

<table>
<thead>
<tr>
<th>Scale values</th>
<th>Text</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (1)</td>
<td>dormant winter bud (late flushing)</td>
</tr>
<tr>
<td>2 (3)</td>
<td>bud-burst (first green is visible) (intermediate)</td>
</tr>
<tr>
<td>3 (5)</td>
<td>leaves are fully expanded (early flushing)</td>
</tr>
</tbody>
</table>

numbers in brackets correspond to the scale mentioned under a)
Pros and cons of the described protocols

a) Pros

- There is a lot of good experience in using this protocol, the scale is in practice. The results of existing accessments can only be converted backwards with losses in a few steps (see Table 3.12.1) not vice versa.
- it is possible to aggregate some steps for comparing assessments (Table 3.12.1),
- this scheme has been discussed among a broad range of foresters (e.g., Treebreedex).

Table 3.12.1: Assessment schemas used for scoring flushing at oak field trials

<table>
<thead>
<tr>
<th>3-step</th>
<th>5-step</th>
<th>5-step BBCH</th>
<th>6-step</th>
<th>8-step</th>
</tr>
</thead>
<tbody>
<tr>
<td>1= late flushing</td>
<td>1= dormant winter bud</td>
<td>1=00= buds are closed and covered in brown scales</td>
<td>1= dormant winter bud</td>
<td>0=dormant winter buds</td>
</tr>
<tr>
<td>2= buds expanding</td>
<td>2=01= the majority of leaf buds have started to expand</td>
<td>2= buds swollen</td>
<td>1=buds swollen</td>
<td></td>
</tr>
<tr>
<td>3= bud-burst (first green is visible)</td>
<td>3=03= the majority of leaf buds have finished expanding and are ready to open</td>
<td>3= buds begin to open</td>
<td>2=buds swollen and elongated</td>
<td></td>
</tr>
<tr>
<td>2= intermediate</td>
<td>4= leaves are flushing</td>
<td>4=07= start of budburst and leaf growth</td>
<td>4= individual small leaves are visible</td>
<td>3=buds burst, first green is visible</td>
</tr>
<tr>
<td>4= buds visible</td>
<td>5= leaves are fully expanded</td>
<td>5=09= the tips of the first leaves are clearly visible</td>
<td>5= leaves are big, but still folded</td>
<td>6=leaves are big, but still folded, main shoot starts to grow</td>
</tr>
</tbody>
</table>

Photos: E. N. Chesnoiu
b) Cons

- It is not always easy to catch the right time to have a good differentiation between the genetic units in flushing.
- Difficulties in choosing proper term of assessment,
- possible subjective influence,
- requires trained staff.

References


3.13 Ash specific protocols

Core group: Joukje Buiteveld (ALTERRA), Arnaud Dowkiw (INRA), Ecaterina Nicoleta Chesnoiu (ICAS), Bart de Cuyper (VLAGEW)

3.13.1 Flushing (bud burst)

Bud burst is the period of the initiation of growth from a bud till elongated shoots with expanded leaves. The trait is under strong genetic control (high heritability) in ash and is influenced by environmental factors and tree health conditions. The most important environmental factors influencing the timing of flushing are spring temperature and winter chilling. Data from provenance trials indicate large differences in flushing time between provenances. In general, northern and western provenances flush later than southern and eastern ones. Also, provenances from high elevations flush later than the ones from low elevations. There is a strong positive correlation between flushing dates between the years. Flushing is an important adaptive trait. Early flushing provenances/trees are prone to damage from late spring frost. This damage may reduce the growth of the tree and affect the tree’s fitness and stem form. There is evidence from more recent studies that flushing is correlated with susceptibility to ash dieback, and that late-flushing trees are more susceptible to infection by fungus.

Description of the assessment in general

Evaluation of bud burst dynamics is based on observations over a period of time with a scoring system describing the different stages for the development of the bud and unfolding of the leaves. It will be assessed on single trees. In principle the terminal bud is scored in young trees up to a height of 5m (average height of a trial series). For older trees (height above 5m) it is difficult to score the single terminal buds. Here a stage is reached when 50% of the crown meets the described criteria of a specific developmental stage (conform BBCH recommendations).

Depending on the research objectives/available resources different ways of assessment are possible:

- Observation only once in spring. The time is chosen so that a maximum variation of the stages occurs in the population of trees to be studied.
- (Julian) day when a particular developmental stage is reached.
- Observation of every tree at steady intervals (of 3 days or a week) during the whole flushing period (gives better insight in the dynamical aspects of bud burst behaviour during the whole observation period).

Each tree is preferably scored on a single day.
Scoring scheme

For young trees of a trial site up to 5 m, a 5-point scale is recommended:

<table>
<thead>
<tr>
<th>Scale values</th>
<th>Text</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>dormant bud</td>
</tr>
<tr>
<td>2</td>
<td>swelling of bud, slight greening of bud scales</td>
</tr>
<tr>
<td>3</td>
<td>buds begin to burst, first green visible</td>
</tr>
<tr>
<td>4</td>
<td>bud burst, petioles of leaves visible, no lengthening of twig</td>
</tr>
<tr>
<td>5</td>
<td>bud burst, petioles of leaves visible, twig has started lengthening, leaves are fully expanded</td>
</tr>
</tbody>
</table>

For older trees (above 5 m) a 3-point scale is recommended:

<table>
<thead>
<tr>
<th>Scale values</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (1)</td>
<td>dormant bud</td>
</tr>
<tr>
<td>2 (3)</td>
<td>buds begin to burst, first green visible</td>
</tr>
<tr>
<td>3 (5)</td>
<td>bud burst, petioles of leaves visible, twig has started lengthening, leaves are fully expanded</td>
</tr>
</tbody>
</table>

(numbers in brackets correspond to the 5-step scale for smaller plants)

Photos: INRA
D2.1 – Common protocols and reference standards for selected traits and species

Pros and Cons

a) Pros

The scoring system covers the total range of developmental stages described, including shoot elongation.

It is quick and simple.

b) Cons

Timing of observation is important to record maximum variation in the trial.

References


3.14 Poplars specific protocols

Core group: Marijke Steenackers (VLAGEW), Sven MG de Vries (ALTERRA), Catherine Bastien (INRA), Alain Berthelot (FCBA), Lorenzo Vietto, Fulvio Ducci (CRA), Volker Schneck, Mirko Liesebach (vTI now THÜNEN)

3.141 Phenology

3.141.1 Bud burst

In springtime, when air temperatures rise to optimal levels, the buds swell and new leaf tissue pushes out of the protective scales. This process is called bud burst or bud break. The objective of the assessment of the earliness of vegetative budbreak is an indirect selection for resistance to spring late frosts.

Description of the assessment

Bud break is evaluated on terminal shoot at two or three years in nursery test with a 6 classes score. The most important stage associated to frost damages is stage 3 (see scoring scheme). Good correlation between nursery stage and adult stage in plantation have been observed.

Bud break earliness is assessed at two year old trees in nursery test on terminal buds of main stem or codominant shoots (coppice). When done at only one date, the assessment of bud break with this 6 classes score is optimum when about 50 % of trees could be scored 3 or more. Optimal evaluation of bud break earliness is obtained with an average score collected on three successive dates (date 1: 25 % of trees have reached score 3 or more, date 2: 50 % of trees have reached score 3 or more, date 3: 75 % of trees have reached score 3 or more.

Scoring scheme

<table>
<thead>
<tr>
<th>Scale values</th>
<th>Text</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Dormant bud completely enveloped by the scales (= perulae)</td>
</tr>
<tr>
<td>2</td>
<td>Bud swelling with scales slightly diverging showing a narrow yellow margin; presence of one or more droplets of balsam</td>
</tr>
<tr>
<td>3</td>
<td>Bud sprouting, with tips of the small leaves emerging out of the scales</td>
</tr>
<tr>
<td>4</td>
<td>Buds completely opened with leaves still clustered together; scales still present</td>
</tr>
<tr>
<td>5</td>
<td>Leaves diverging with their blades still rolled up; scales may be present or absent</td>
</tr>
<tr>
<td>6</td>
<td>Leaves completely unfolded (but smaller in size than mature ones); lengthening of the axis of the shoot evident; scales absent</td>
</tr>
</tbody>
</table>
**Pro and con**

This protocol is suitable to measure quickly a large number of trees.

**Reference clones**

<table>
<thead>
<tr>
<th>Budflush phenology</th>
<th>Standard clones</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early budflush</td>
<td>Flevo, I-214, Soligo</td>
</tr>
<tr>
<td>Medium budflush</td>
<td>Alcinde, Catherine aanvulleren</td>
</tr>
<tr>
<td>Late budflush</td>
<td>Koster, Dorskamp, Blanc du Poitou, Triplo</td>
</tr>
</tbody>
</table>

**References**


**3.141.2 Bud set**

**Definition**

The objective of the assessment bud set lateness is an indirect selection for resistance to early frosts in autumn. Date of bud set is evaluated on terminal shoot at two years in nursery test with the following 7-classes-score (protocols B or C). The most important stage is stage 1,5; stage 2,5 correspond to cessation of elongation. Length of photoperiod and average temperature are known to influence jointly date of growth cessation and date of bud set.
Description of the assessment

Date of bud set is evaluated on terminal shoot at two years in nursery test with the 7-classes-score (protocols B or C) given below. The most important stage is stage 1,5; stage 2,5 correspond to cessation of elongation. Length of photoperiod and average temperature are known to influence jointly date of growth cessation and date of bud set.

When done at only one date, the assessment of bud set with this 7-classes-score is optimum when about 50 % of trees could be scored 1.5 or less. Optimal evaluation of date of bud set is obtained with an average score collected on three to five successive dates.

Scoring scheme

<table>
<thead>
<tr>
<th>Scale values</th>
<th>Text</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (0 in figure)</td>
<td>Apical bud red brown</td>
<td></td>
<td>No more rolled leaves</td>
</tr>
<tr>
<td>2 (0,5)</td>
<td>F1 fully stretched comparable to older leaves apical but of older leaves; apical bud of fully closed between green and red</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 (1)</td>
<td>F1 fully stretched with ‘juvenile aspect’; F2 comparable to older leaves apical bud not fully closed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 (1,5)</td>
<td>F2 fully stretched transition to bud structure</td>
<td></td>
<td>Presence of rolled leaves</td>
</tr>
<tr>
<td>5 (2)</td>
<td>F2 fully stretched no bud structure</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 (2,5)</td>
<td>2 rolled-up young leaves at equal height</td>
<td>2 rolled-up young leaves at equal height</td>
<td></td>
</tr>
<tr>
<td>7 (3)</td>
<td>&gt;2 rolled-up young leaves</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
D2.1 – Common protocols and reference standards for selected traits and species

Bud-set Score Card *Populus nigra*

<table>
<thead>
<tr>
<th>Trait Description</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apical shoot fully growing &gt; 2 rolled-up young leaves</td>
<td>3</td>
</tr>
<tr>
<td>Last leaves still rolled-up</td>
<td>2,5</td>
</tr>
<tr>
<td>Last leaves at equal height</td>
<td>2</td>
</tr>
<tr>
<td>Stem internodes become shorter</td>
<td>1,5</td>
</tr>
<tr>
<td>Shoot internode growth reduced</td>
<td></td>
</tr>
<tr>
<td>2nd leaf fully stretched</td>
<td></td>
</tr>
<tr>
<td>2nd leaf bright green</td>
<td></td>
</tr>
<tr>
<td>Transition from shoot to bud structure</td>
<td></td>
</tr>
<tr>
<td>Bud not fully closed</td>
<td>1</td>
</tr>
<tr>
<td>Buds scales predominantly green</td>
<td>0,5</td>
</tr>
<tr>
<td>No more rolled-up leaves</td>
<td></td>
</tr>
<tr>
<td>Apical bud fully closed</td>
<td></td>
</tr>
<tr>
<td>Colour between green and red, stipules of the two last leaves still green</td>
<td></td>
</tr>
<tr>
<td>Apical bud red-brown</td>
<td></td>
</tr>
</tbody>
</table>

**Balsam**

- Lots of balsam covering multiple internodes
- Apical shoot fully covered with balsam, also present in subsequent nodes
- Top wet and covered, mostly absent in subsequent nodes
- Leaf well visible, no longer fully covered with balsam
- Balsam sticky and shiny
- Balsam fully dried, no balsam visible

---

Note: The scores may not always be applicable. It can be scored separately whether last leaf (or leaf tip) is expressed from the bud.
Pro and con

These protocols are suitable to measure quickly a large number of trees.

References


3.142 Melampsora larici-populina

Melampsora larici-populina is the most common and dangerous Eurasian rust, and requires and alternate host „Larix“ to complete its life cycle. Uredinia, telia and basidia are formed on poplar, spermogonia and aecia are formed on larch. Uredinia commonly develop on the lower surface of leaves, giving rise to yellow or necrotic spots and flecks on the upper surface. Severely affected trees are defoliated prematurely, reducing growth potential and predisposing them to environmental stresses and invasion by secondary damaging agents. Early leaf fall also increases the risk for winter injury, dieback and infection by secondary pathogens. The disease is generally controlled by planting tolerant cultivars or clones to the local populations of rust. The potential evolution of new rust pathotypes underscores the critical need of monitoring poplar rust populations.

Description of the assessment in general

Different evaluation tests are used to evaluate susceptibility to Melampsora larici-populina: (1) qualitative resistance tests under artificial inoculation with known reference strains; (2) quantitative resistance tests on leaf disks inoculated with known reference strains and (3) susceptibility tests in field experiments under natural inoculation associated to characterization of the pathogen population.

(1) Protocol for qualitative resistance tests under artificial inoculation

The objective of qualitative resistance tests is to identify qualitative resistances inherited from P. deltoides and overcome by at least one of the eight known M. larici-populina virulences. Leaf disks collected on disease-free plants are inoculated artificially at high density (40000 spores/ml) with a solution of mono-uredinial strains of known combination of virulences (or pathotype). A minimum of 6 leaf disks sampled from the 5th to the 8th unrolled leaf below the apex are inoculated per tested genotype. After inoculation, leaf disks are maintained in climatic chamber at a temperature between 17°C and 20°C with a 16-hours photoperiod. The response observed 14 days after inoculation is presence/absence of
uredinia or any leaf decoloration or any leaf necrosis. A genotype is considered as susceptible to the strain studied when a minimum of one leaf disk presents sporulated uredinia. A genotype is considered as resistant to the strain studied when all the inoculated leaf disks are free of any symptoms.

The M. larici-populina strains are chosen in order to represent the eight known virulences. For each inoculation test and each strain, the following differential genotypes are used to confirm the pathotype of the strain used:

<table>
<thead>
<tr>
<th>Type botanique</th>
<th>Differential Clones</th>
<th>List of Mlp virulences for which a compatible response of the differential clone is observed</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. × canadensis</td>
<td>Robusta</td>
<td>1,2,3,4,5,6,7,8</td>
</tr>
<tr>
<td>P. × canadensis</td>
<td>Ogy</td>
<td>1</td>
</tr>
<tr>
<td>P. candicans</td>
<td>Aurora, Grimminge</td>
<td>2</td>
</tr>
<tr>
<td>P. × canadensis</td>
<td>Brabantica</td>
<td>3</td>
</tr>
<tr>
<td>P. × generosa</td>
<td>Unal</td>
<td>4</td>
</tr>
<tr>
<td>P. × generosa</td>
<td>Rap</td>
<td>5</td>
</tr>
<tr>
<td>P. deltoides, P. × generosa</td>
<td>87B12, 84B09</td>
<td>6</td>
</tr>
<tr>
<td>P. × generosa</td>
<td>Beaupré</td>
<td>7</td>
</tr>
<tr>
<td>P. ×x generosa</td>
<td>Hoogvorst, hazendans</td>
<td>8</td>
</tr>
</tbody>
</table>

(2) Protocol for qualitative resistance tests under artificial inoculation

The objective of quantitative resistance tests is to rank poplar genotypes for their ability to limit infection by M. larici-populina in absence of qualitative resistance. The three components of quantitative response associated to pathogen fitness are measured on 3 cm diameter excised leaf disks inoculated with a reference mono-uredinial strain. The leaf disks are floated upside down on distilled water in polycarbonate 6-well cell culture plates. The inoculum suspension concentration was 30 mg/liter : 3 mg urediniospores in 100 ml distilled water added with 0.5 mg agar. Sprayed suspension volume per leaf surface ratio was 20 ml/m². The disks were kept for 14 days under growth chamber controlled conditions (17 °C, 16 h photoperiod, 30 µE.m-2.s-1).

The following three components of quantitative resistance are observed on each leaf disk:

- the **latent period**, measured on a half a day basis, which corresponds to the date when the first sporulating uredinia can be observed.
- the **number of sporulating uredinia** 13 days after inoculation
- The **average uredinia size score** (US$_{scale}$) 14 days after inoculation. This score uses a 1 to 5 scale defined according to the size variability observed in the experiment. To obtain an objective assessment of uredinia size, 25 leaf disks per score (75 for the whole tests) are sampled for image analysis. Average uredinia surface in mm² is measured and calculated on all sporulating uredinia of the inoculated leaf disk.
(3) Protocol to evaluate susceptibility to *Melampsora larici-populina* leaf rust under natural infection in field tests

Susceptibility to *Melampsora larici-populina* leaf rust under natural infection can be evaluated by two protocols, dependent on the time of the year: protocol A) the maximum infected leaf score and protocol B) the global sanitary tree score.

**Protocol A:**

The **maximum infected leaf score** quantifies the infection level of the most infected leaf on the terminal shoot. This observation is done quite early, between end of June and mid-July when the infection could be considered as homogeneous in the field experiment. At this time of infection, the most infected leaves are still on the trees.

**Protocol B:**

The **global sanitary tree score** quantifies the damages (infection, necrosis, leaf fall and even plant death) at the plant level. This observation is done late in season, between mid-August and mid-September just before growth cessation.

**Pros and cons**

The protocols are easy to carry out on a large number of trees per time unit (several hundreds per day).

The global sanitary tree score could take into account other damages than those directly linked to *Melampsora larici-populina* infection.

**Reference genotypes**

The following standard clones with known levels of susceptibility to *Melampsora larici-populina* to populations including the eight known virulences need to be included in field experiments:

<table>
<thead>
<tr>
<th>Susceptibility level to Mlp in field experiments in presence of all virulences</th>
<th>Standard clones</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very low susceptible</td>
<td>Alcinde, Lena, Bakan, Skado, Soligo, Triplo, Villafranca</td>
</tr>
<tr>
<td>Low susceptible</td>
<td>87B12, Dvina, I-214, Flevo, Dorskamp, Koster, Fritz Pauley, Trichobel, Grimminge, Vesten</td>
</tr>
<tr>
<td>Susceptible</td>
<td>Robusta, Ogy, Brabantica, Unal, 84B09, Blanc du Poitou, I-45/51, Raspalje, Vereecken</td>
</tr>
<tr>
<td>Very susceptible</td>
<td>Aurora, Rap, Beaupré, Hoogvorst</td>
</tr>
</tbody>
</table>

It is important to characterize the pathogen population responsible of the natural infection observed in field tests. This characterization is obtained by molecular analysis to confirm the pathogen species and by isolation and pathotype determination of about 100 distinct uredinia sampled on different leaves of the ‘Robusta’ standard clone.
Scoring schemes

**Protocol A**: Maximum infected leaf score (1-8); end of July (mid-infection)
Source: INRA-FCBA

<table>
<thead>
<tr>
<th>Scale values</th>
<th>Text</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>No uredinia</td>
</tr>
<tr>
<td>7</td>
<td>Few difficult to detect</td>
</tr>
<tr>
<td>6</td>
<td><em>Uredinia easy to detect</em> but not joined</td>
</tr>
<tr>
<td>5</td>
<td>Joined uredinia covering <em>less than 10 %</em> of the leaf area</td>
</tr>
<tr>
<td>4</td>
<td>Joined uredinia covering <em>between 10 % and 25 %</em> of the leaf area</td>
</tr>
<tr>
<td>3</td>
<td>Joined uredinia covering <em>between 25 % and 50 %</em> of the leaf area</td>
</tr>
<tr>
<td>2</td>
<td>Joined uredinia covering <em>between 50 % and 75 %</em> of the leaf area</td>
</tr>
<tr>
<td>1</td>
<td>Joined uredinia covering <em>more than 75 %</em> of the leaf area</td>
</tr>
</tbody>
</table>

**Protocol B**: Global sanitary tree score (1-7); mid-August till mid-September
Source: INRA-FCBA, INBO

<table>
<thead>
<tr>
<th>Scale value</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>0 uredinia on the plant (qualitative resistance)</td>
</tr>
<tr>
<td>6</td>
<td>Few uredinia hard to detect</td>
</tr>
<tr>
<td>5</td>
<td>Many uredinia per leaf but no decoloration neither necrotic zones</td>
</tr>
<tr>
<td>4</td>
<td>Many leaves infected but still green. Limited decolorated or necrotic zones, no defoliation</td>
</tr>
<tr>
<td>3</td>
<td>Numerous leaves highly infected with decoloration and necrotic areas, significant defoliation but between 1/2 and 1/3 of green leaves on the top of the plant.</td>
</tr>
<tr>
<td>2</td>
<td>Most of leaves have decolorations and necrotic areas, important defoliation (30%-50%), less than ¼ of remaining leaves are still green.</td>
</tr>
<tr>
<td>1</td>
<td>Important defoliation (&gt;50 %), no more significant elongation, only few remaining leaves on the top</td>
</tr>
</tbody>
</table>
3.143 Marsonia brunea

Marssonina brunnea is a damaging foliar pathogen of poplar. Severe attacks on susceptible clones can cause premature defoliation, resulting in significant growth reduction, dieback, and predisposition to secondary organisms and abiotic stress. Marssonina brunnea causes dark brown, circular to angular leaf spots that usually measure about 1 mm in diameter. On highly susceptible clones, lens-shaped lesions develop on petioles and current-year shoots. Damage can be especially severe where poplar species and clones are selected without regard to their susceptibility to the disease.

Description of the assessment in general

This protocol has been adapted from the international protocol defined in 1975 by the International Poplar Commission and optimized by INRA-FCBA. A special field experiment is established in nursery for a minimum of three years of observation at very high plantation density (0.4 to 0.6 m in the line, 1.5 to 2m between lines) to favor contamination. It is also recommended to plant at regular spacing a very susceptible reference clone such as Magister Géant or I-214 or Tardif de Champagne. To favor contamination, infected leaves can be attached on the very susceptible reference clone. Between three and five reference clones from the following list are included in the test:

<table>
<thead>
<tr>
<th>Botanic type</th>
<th>Very susceptible reference clones</th>
<th>Susceptible reference clones</th>
<th>Resistant/Tolerant reference clones</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. deltoides</td>
<td>Lena</td>
<td>Lux</td>
<td>Dvina</td>
</tr>
<tr>
<td>P. × canadensis</td>
<td>Magister géant, I-45/51, Tardif de Champagne</td>
<td>I-214, Robusta, Brenta, Mella, Triplo, Lambro, Soligo, Blanc du Poitou</td>
<td>Dorskamp, Polargo, Lux</td>
</tr>
<tr>
<td>P. trichocarpa</td>
<td></td>
<td>Fritzi Pauley</td>
<td></td>
</tr>
<tr>
<td>P. × generosa</td>
<td>Hazendans, Grimminge, Raspalje</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other interspecific hybrids</td>
<td></td>
<td></td>
<td>Taro</td>
</tr>
</tbody>
</table>

Source: INRA-FCBA
Response to contamination is scored twice during the growing season: once early in June and once mid August just before significant leaf fall.

**Scoring scheme**

On the main stem of each plant, all leaves are scored with the following scoring system:

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>Leaf free of disease</td>
</tr>
<tr>
<td>3</td>
<td>Between 1 and 11 acervulae on the leaf</td>
</tr>
<tr>
<td>2</td>
<td>Between 11 and 100 acervulae on the leaf</td>
</tr>
<tr>
<td>1</td>
<td>More than 100 acervulae on the leaf</td>
</tr>
</tbody>
</table>

Relative frequencies of the 4 leaf scores at an individual tree level are then calculated to estimate genotype susceptibility to *Marssonina brunnea*.

**Pros and cons of the described protocol**

This protocol is very detailed but not suitable to measure quickly a large number of trees.

**3.144 Xanthomonas populi**

The bacterium *Xanthomonas populi* is one of the main poplar pathogens throughout much of Europe.

Susceptibility of clones is tested by artificial inoculation on two years old plants in the field. *Xanthomonas populi* isolates used for inoculation can be obtained from the collection CFBP at INRA-Angers (FR) or from INBO collection, Geraardsbergen (BE). Isolates are reactivated and multiplicated at 20°C in Petri dishes filled with a LPGA medium. The inoculation suspension is adjusted to $10^8-10^9$ cells per ml just before inoculation.

Two or three transversal incisions are made on tree main stem between 0,80 and 1,20m (about 10mm large and 2mm long). 25 µl of the inoculation suspension is deposited on the fresh wound. The inoculation is done in early September, in the period just before closing of the buds.

Response to inoculation is measured one and two years after inoculation at each inoculation point thanks to two different traits:
- Longitudinal canker extension (mm)
- Girdling Index score (0-5):
<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>No symptom</td>
</tr>
<tr>
<td>5</td>
<td>canker is developed along less than 20 % of stem circumference</td>
</tr>
<tr>
<td>4</td>
<td>canker is developed along between 20 % and 40 % of stem circumference</td>
</tr>
<tr>
<td>3</td>
<td>canker is developed along between 40 % and 60 % of stem circumference</td>
</tr>
<tr>
<td>2</td>
<td>canker is developed along more than 60 % of stem circumference</td>
</tr>
<tr>
<td>1</td>
<td>total girdling of tree stem with associated mortality of the apex</td>
</tr>
</tbody>
</table>

The following reference clones are used for comparison:

<table>
<thead>
<tr>
<th>Botanic type</th>
<th>Very susceptible to race1</th>
<th>Susceptible to race1</th>
<th>Resistant to race1</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. x generosa</em></td>
<td>S.6-2</td>
<td></td>
<td>Boelare, Raspalje, Beaupré</td>
</tr>
<tr>
<td><em>P. trichocarpa</em></td>
<td></td>
<td>S.3-31</td>
<td>Fritzi Pauley</td>
</tr>
<tr>
<td><em>P. x canadensis</em></td>
<td>Donk, I-45/51</td>
<td>Blanc du Poitou, Dorskamp</td>
<td>I-214, Koster</td>
</tr>
</tbody>
</table>

**Pros and cons of the described protocol**

Selection for susceptibility to *Xanthomonas populi* is a long-term test, as it is done at 1 and 2 years after artificial inoculation on 2-year-old trees. The protocol itself is easy to perform.

**References**

3.15 Wild Cherry specific protocols

Core group: Fulvio Ducci, Roberta Proietti (CRA), Frédérique Santi (INRA), Joukje Buiteveld (ALTERRA), Bart de Cuyper, Marijke Steenackers (VLAGEW)

3.151 Phenology

Definition
Phenology is an important aspect of adaptation. It is the study of the timing of periodic phenomena such as bud break, flowering, growth initiation, growth cessation, water flows, cambial and physiological activity, especially as related to seasonal changes in temperature, photoperiod, etc. (Nanson 2004).

Phenology traits are conditioned by biological and environmental factors (chilling requirements, forcing temperatures, seasonal changes, etc.) necessary for launching the processes, but they are also under strong genetic control. They are important selection criteria as they might condition stem form in the case of stress, frost damage (mostly late frost), but they also affect growth potential in relation to the growth season length. Then they are directly related to the growth and to the tree architecture.

Experimental data from already performed surveys show the adaptive meaning of this group of specific traits. Indeed, clones and provenances are strongly influenced in phenology by their origin. Phenology traits are therefore practically relevant indicators of the adaptability and adaptedness of forest trees and are useful to study the effect to global change. A series of assessments on the same individuals over more years allows conclusions about the climate development. It might be useful to identify extreme standard materials which could be used as references in trials to alert for phenology observations. Preferably it should be material shared by as many test sites as possible.

3.151.1 Flushing (bud break)

Definition
Bud break is the period spanning from dormant bud up to shoot elongation. It is conditioned by internal (genetic) and environmental factors (chilling requirements and forcing temperatures).

Description of the assessment in general
A subjective scoring system is commonly used. But in any case, due to the size of trees, different methods need to be adopted depending on whether young or adult trees have to be monitored.

In young trees (up to 2.5 m), observation usually concerns the terminal buds, while in adult and taller trees (more than 2.5 m) the terminal bud of lateral branches or the same portion of the crown should be monitored. Attention should be also paid to the acropetal or basipetal sense of phenology in the crown. I.e., in wild cherry, phenology proceeds in an
acropetal way. That means that the crown should be ideally divided in 3 sectors to keep the same method in all the monitored trees (Figure 3.15.1).

Figure 3.15.1: For phenological surveys in adult trees, the crown should be ideally divided into 3 sectors.

The monitoring has to be performed in each sector and located in the same aspect. A phenological stage of a sector is reached when 50 % of buds show the characteristics describing a specific score.

A sufficient number of trees to perform statistical analysis must be surveyed. When clones are considered, the number of plants per clone can be sensibly reduced to 3 – 5, while when progenies or provenances are monitored, the number can vary between 20 and 30 or more trees.

Based on scientific questions and on funding resources, monitoring can be either conducted following a regular timing or just occasionally. The most common criteria are:

- **twice a week** during all the flushing period. Each tree is monitored at each assessment;
- **at a given periodicity** (days/week) during the flushing period by assessing only trees having reached a given score or all trees at different scores;
- **only once or twice** during the flushing season when distances are an important limiting factor for surveys.

The monitoring season should start soon enough to capture early stage and end late enough to catch the last stage. Each flushing assessment is to be carried out on the same day, or with a difference of one day maximum.
To reduce errors due to a subjective attribution of scores, the same staff should be involved in the survey.

Monitoring can be carried out either in controlled or semi controlled environments (growth chamber, greenhouse) or in field (nursery, plantation). Anyway in field trials and plantations in general it is suggested that the monitoring start at least two years later in order to reduce the acclimation effect. The observation will stop when all trees have reached the last stage.

**Scoring scheme**

The scoring system is the same for either in young and adult trees, but in the first case the assessment is carried out on the apical bud, while in the second one it is on the branch apical buds within a sector. A 5-step-scale is considered (Table 3.15.1; Figures 3.15.2 and 3.15.3).

**Table 3.15.1. 5-step-scale used for wild cherry flushing monitoring**

<table>
<thead>
<tr>
<th>Scale values</th>
<th>Text</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Buds not active, scales brown and closed (dormant winter bud)</td>
</tr>
<tr>
<td>2</td>
<td>Buds expanding, scales start to separate</td>
</tr>
<tr>
<td>3</td>
<td>Bud-burst (first green is visible)</td>
</tr>
<tr>
<td>4</td>
<td>Leaves are flushing</td>
</tr>
<tr>
<td>5</td>
<td>Leaves are fully expanded and new sprouts are in elongation</td>
</tr>
</tbody>
</table>

**Figure 3.15.2: 5-step scale for young trees**
For *P. avium* an integrated approach for phenology assessment can be also considered that combines bud break, flowering and ripening of fruits (Ducci et al. 2012). Scores used can be compared with phenological growth stages defined by BBCH scale (Meier 1997, Meier et al. 2009) for stone fruit (Table 3.15.2 and Figure 3.15.4).
Table 3.15.2: BBCH Principal growth Stage 0 (sprouting/Bud development) and Stage 1 (leaf development) and BBCH-identification keys of stone fruit.

<table>
<thead>
<tr>
<th>BBCH growth stage</th>
<th>Text</th>
</tr>
</thead>
<tbody>
<tr>
<td>00</td>
<td>Dormancy: leaf buds and the thicker inflorescence buds closed and covered by dark brown scales</td>
</tr>
<tr>
<td>03</td>
<td>End of leaf bud swelling: scales separated, light green bud sections visible</td>
</tr>
<tr>
<td>10</td>
<td>First leaves separating: green scales slightly open, leaves emerging</td>
</tr>
<tr>
<td>11</td>
<td>First leaves unfolded, axis of developing shoot visible</td>
</tr>
<tr>
<td>19</td>
<td>First leaves fully expanded</td>
</tr>
</tbody>
</table>

Figure 3.15.4: BBCH score scale for stone fruit. Scores from 00 to 31 will be considered in this framework.

This scoring system can be expensive in terms of time/personnel (a person is on average able to sample about 200 trees/day according to the size of trees) and it is suggested to be applied within collections, with special attention to reference clones.
1.151.2 Leaf senescence

Definition

Leaf senescence corresponds to the period when progressive changing of leaf color is observed. Leaf color can be characterized by high heritability, with special regard to clonal materials. Under local conditions relatively homogeneous color can be an important source of information for recording adaptive variation.

Spatial micro-environmental variations, extreme environmental events (e.g., drought), the plant health conditions (bacterial/fungal spots), the sampled leaf amount and acropetal/basipetal senescence (thus the crown has to be divided into 3 sectors) represent sources of measurement error.

Description of the assessment in general

For leaf senescence (yellowing) a subjective scoring system allows trees to be classified according to a grid representing different stages of tree yellowing. Observation is best done on small trees like in a nursery (but transplantation shock could be a source of error) or in field experiments (with trees of heights up to 3-4 m). Generally the whole crown of trees is observed.
**Scoring scheme**

Assessment of senescence *via a scoring system* is generally used. It is a very subjective method. The organization of the survey is the same as for flushing. The senescence progress within the crown is acropetal, starting the first color variation from the bottom towards the tip. So, even in this case observations have to be divided among the three crown sectors or focused on only one of them.

The BBCH system allows a subjective evaluation of color phases to be avoided and this system is again suggested. The BBCH growth Stage 9 (Senescence, beginning of dormancy) for stone fruit is reported in Table 3.15.3.

Table 3.15.3: Principal growth BBCH Stage 9: Senescence, beginning of dormancy.

<table>
<thead>
<tr>
<th>BBCH growth stage</th>
<th>Text</th>
</tr>
</thead>
<tbody>
<tr>
<td>91</td>
<td>Shoot growth completed; foliage still fully green</td>
</tr>
<tr>
<td>92</td>
<td>Leaves begin to discolor</td>
</tr>
<tr>
<td>93</td>
<td>Beginning of leaf fall</td>
</tr>
<tr>
<td>95</td>
<td>50% of leaves discolored or fallen</td>
</tr>
<tr>
<td>97</td>
<td>All leaves fallen</td>
</tr>
<tr>
<td>99</td>
<td>Harvested product (not to be considered in the framework of T4F)</td>
</tr>
</tbody>
</table>

A further possibility to evaluate senescence is offered by the use of colorimeters. These allow yellowing to be assessed *via quantitative methods*. At the clone level these traits can be highly heritable (0.67 on average for sensu lato heritability). Data can be managed as quantitative traits using a Portable Colorimeter Microflash v4.0. (methodology is described in wood color sheet).

### 3.152 Wood color

**Definition**

*Wood color* is an aesthetic trait, for which special attention is placed in wild cherry and in broadleaves because wood is used to make valuable furniture and cabinetry. Then the evaluation of wood color is useful for both the characterization of reproductive materials and for the wood industry.

**Assessment**

Quantitative parameters of the physical components of the color are evaluated using colorimeters, portable tools currently adopted by the paint or tissue industries for standardizing their production. Colorimeters using standard illuminants (mainly white light illuminants), and recording the reflexed light components, are able to measure basic parameters with very good approximation. Data can be managed as quantitative traits according to usual statistic methods.
Wood color, both of hardwood (duramen) and soapwood can be measured using:
1) **destructive method**, on circular sections of the trunk at different heights (at the base, at breast height and at 3-m);
2) **non-destructive method**, taking cores at the same heights from trees about 15-20 years of age (or > 20cm DBH).

The Colorimeter Portable Microflash v4.0 (Figure 3.15.5) provides data relating to the colorimetric coordinates of the CIEL*a*b* system:
• L*: Luminance: is the variation of the color component along the black to white axis (z);
• a*: is the variation of the color component along the green to red axis (x);
• b*: is the variation of the color component along the blue or yellow axis (y).

Other parameters can be derived from the above mentioned ones, such as: **white index** (expressing the darkness/whiteness of the sample), C* (chroma/saturation; expressing the intensity of the color measured) and h (taint angle; expressing the height of the sample in the color space).

**Methods**

The color measurements (BOURGOIS et al. 1991) are carried out in the CIE- L*a*b* colour space using spectrophotometers with a standard illuminant D65 and 10° circular illumination.

The color coordinates are calculated from the reflection data on the sample surface. In this color range, a colour is defined by its Cartesian chromatic coordinates: lightness L* (varies from 0 - black to 100 - white) and a* and b* (these coordinates define the chromatic plane; negative values of a* indicate green, while positive values indicate red; negative values of b* indicate blue, while positive values indicate yellow). The difference in chromaticity is defined as: \( \Delta C = [(\Delta a^*)^2 + (\Delta b^*)^2]^{1/2} \).

The CIE L*a*b* system produces a fair correlation with the visual perceptions (CHRISMENT 2000).

Concerning the color development during irradiation, it is useful to present the data in the CIE L*C*h* system which is more uniform (closer to the psycho-sensory assessment). The
color development can be derived simply from the CIE L*a*b* system by changing the Cartesian coordinates to cylindrical ones in the chromatic plane [a*, b*]. The Chroma (concentration or degree of color saturation) is defined then by $C^* = (a^*^2 + b^*^2)\frac{1}{2}$ and the hue (saturation angle) $h = \arctan \left(\frac{b^*}{a^*}\right)$.

The mean values of chromatic coordinates are calculated from measurements at different points on the radial surface of the sample (30x30 x10 mm, LxTxR).

Tools/Equipment: quantitative or relative data can be recorded with spectrophotometers by different brands, i.e.,:
- Spectrocolorimeter Datacolor *Portable Microflash v4.0, with a measurement opening of 3 mm,
- Konica Minolta spectrophotometer CM-2600 ® with D65 illuminant, a standard angle of 10° and a measurement opening of 8 mm.

References


3.16 Broadleaves non-species specific protocols

Core group (broadleaves species leader):
- **Mirko Liesebach** (vT now THÜNEN; beech),
- Joukje Buiteveld (ALTEGRA; common ash),
- Fulvio Ducci, Roberta Proietti (CRA; wild cherry),
- Marek Rzonca (IBL; oak),
- Marijke Steenackers (VLAGEW; poplar),
- Sven M.G. de Vries (ALTEGRA; poplar)

The non-species specific traits for broadleaves describe the quality. These traits have an effect on the economic value of the final product. The final product is the trunk, particularly the lower part of the trunk.

### 3.16.1 Straightness

**Definition**

A non-species specific trait for broadleaves is **straightness**. Stem straightness is under genetic control and can be affected by environmental conditions.

Results from progeny trials show large differences in the frequency of straightness classes.

**Description of the assessment in general**

Scoring straightness should be started when the trial has a mean height of 5 m. From then on straightness can be assessed without an upper height limit.

The assessment has to be concentrated on about ¾ of the tree height beginning from the ground. That means the straightness in the crown is not taken into the assessment. The significance of the assessment increases with the age which is correlated with the height of the tress.

In case of forked stems, only the trunk below the deepest forking point is evaluated. The assessment can be carried out during the whole year, particularly in older trials.
**Scoring schema**

A 5-step-scale is recommended:

<table>
<thead>
<tr>
<th>Scale values</th>
<th>Text</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>absolutely straight stem</td>
</tr>
<tr>
<td>4</td>
<td>fairly straight (in one direction slightly crooked)</td>
</tr>
<tr>
<td>3</td>
<td>slight to moderate bends in different directions</td>
</tr>
<tr>
<td>2</td>
<td>moderate to strong bends</td>
</tr>
<tr>
<td>1</td>
<td>no straight stem</td>
</tr>
</tbody>
</table>

**Pros and cons of the described protocols**

a) Pros

There is good experience in using the protocols. In several investigations a simplified version (only 3 steps were assessed: 1, 3, 5).

b) Cons

There is a slight risk that the straightness of a thinner tree will be classified better than those of a thicker tree.

**References**


3.162 Forking

Definition
A non-species specific trait for broadleaves is forking. Forking is can be caused both under genetic control and as damage to the terminal bud affected by environmental conditions. Forking can result by biotic and abiotic effects, for example frost events and deer browsing, respectively. Results from progeny trials show differences in the frequency of forking classes.

Compared to a ramicorn, a fork has two leaders of equal importance in thickness and length, while a ramicorn is a branch thicker than mean branches, but thinner and usually shorter than the main stem.

Description of the assessment in general
Presence versus absence of a fork is the most straightforward method. According to the height at which a fork appears, the effect can be judged detrimental or not.

Scoring forking should be started when the trial has a mean height of 5 m. From then on forking can be assessed without any height limit.

Scoring schema
For trees up to a mean height of 2 m of a trial series a 5-steps-scale is recommended:

<table>
<thead>
<tr>
<th>Scale values</th>
<th>Text</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>No fork</td>
</tr>
<tr>
<td>4</td>
<td>Branch (ramicorn; thinner than a fork) with a similar angle</td>
</tr>
<tr>
<td>3</td>
<td>Fork(s) only in upper half of the tree height</td>
</tr>
<tr>
<td>2</td>
<td>Fork(s) only in the lower half of the tree height</td>
</tr>
<tr>
<td>1</td>
<td>Forks developed both in upper and in lower half of the tree height</td>
</tr>
</tbody>
</table>
Pros and cons of the described protocols
When felling a tree with a fork, there is a risk that the trunk below the fork will tear. The parts above the forking point are thinner than the trunk below. This means the economic value of the trunks is smaller than a continuous thick trunk from the ground to the crown. An additional counting of the forks is therefore unnecessary.

a) Pro
There is experience in using the protocol to assess forking.

b) Con
It is sometimes difficult to distinguish between the reasons of forking, in particular when a tree has one fork. In the case of many forks in a tree, a genetic effect is presumable. A change from a fork to a ramicorn is fluent.

References
NW-FVA (not published) cited in PÂQUES L 2009: European survey on assessment methods of morphological traits. Treebreedex Doc. 916

3.163 Branch habit

3.163.1 Branch angle
Definition
Branch angle is important for the wood quality and has an effect on economic value. A steep branch is longer in wood then a plain growing branch. Between a steep growing branch and the stem, water can accumulate and can cause rotting.

Description of the assessment in general
Branch angle is defined as the angle between a given branch and the main stem axis. The angle is measured at the insertion point.

Practical assessment: dividing the angle between a rectangular growing branch and the stem into 3 angle classes with the same size.

The scoring schema can be used at any age but preferable beyond the juvenile stage when the tree architecture stabilizes. For the assessment the mean branch angle class of all branches should be regarded.
Scoring schema

<table>
<thead>
<tr>
<th>Scale values</th>
<th>Text</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>&lt;30° (steep)</td>
</tr>
<tr>
<td>2</td>
<td>30°-60°</td>
</tr>
<tr>
<td>3</td>
<td>60°-&gt;90° (plain)</td>
</tr>
</tbody>
</table>

Pro and con of the described protocols

a) Pros

The assessment can be carried out easily.

b) Cons

There is the danger of overestimation in the case of concentrating on a single branch, which is wrongly in focus. The reason for such a branch might be environmental.

3.163.2 Branch thickness

Definition

Branch thickness is important for the wood quality and has a direct effect on the economic value. A thin branch is better for the wood quality. The cutting point is restored soon after natural and artificial pruning.
Description of the assessment in general

Direct measurement is very time consuming. Therefore a 3-step scoring is recommended. The trait branch thickness is provided as a relation of branch thickness to stem diameter. To get a feeling for branch thickness it could be helpful to measure a number of branches.

Scoring schema

3-step scale

<table>
<thead>
<tr>
<th>Scale values</th>
<th>Text</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$\phi_{\text{branch}} / \phi_{\text{stem}} \leq 0.3$ (fine branches)</td>
</tr>
<tr>
<td>2</td>
<td>$0.3 &lt; \phi_{\text{branch}} / \phi_{\text{stem}} &lt; 0.6$ (moderate branches)</td>
</tr>
<tr>
<td>3</td>
<td>$\phi_{\text{branch}} / \phi_{\text{stem}} \geq 0.6$ (thick branches)</td>
</tr>
</tbody>
</table>

Pros and cons of the described protocols

a) Pros

Having the feeling it is easy to assess.

b) Cons

Time is needed to get the feeling for evaluation. An underestimation of thicker trees can happen because thinner trees give the impression of having thinner branches.
3.2 Conifers

3.21 Norway spruce specific protocols

Core group: **Luc Harvengt** (FCBA),  
Jan Kowalczyk, Marek Rzońca (IBL),  
Egbert Beuker (METLA now LUKE),  
Mirko Liesebach (vTI now THÜNEN)

3.211 Flushing

**Definition**

Bud break and bud set are among the most heritable traits of Norway spruce (*Picea abies* [L.] Karst.). Provenance tests show a great variability between populations in flushing. Provenances originating from higher elevations flush earlier than provenances from lower elevations. Also provenances originated in the northern part of the distribution area need a lower amount of temperature sum to flush and therefore, flush earlier than those originated in the south. Genotype’s ranking for bud break is also very stable across sites and years.

**Description of the assessment in general**

Bud break should be assessed separately for each tree in the field.

**Part of the crown to be assessed:** Preferably the top of the crown (light crown) should be visible from one observation point. If this is not possible, then the middle part of the crown is also acceptable. The same part of the crown should be considered for subsequent phenological observations throughout the whole flushing period, as well as for subsequent years.

**Scoring schema**

5-step scale

<table>
<thead>
<tr>
<th>Scale values</th>
<th>Text</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Dormant winter bud</td>
</tr>
<tr>
<td>2</td>
<td>Starts flushing (first green is visible at the tip)</td>
</tr>
<tr>
<td>3</td>
<td>First needles visible (“brush stage”)</td>
</tr>
<tr>
<td>4</td>
<td>New shoot app. 3 cm</td>
</tr>
<tr>
<td>5</td>
<td>New shoot &gt; 4 cm</td>
</tr>
</tbody>
</table>
**Pros and cons of the described protocols**

a) Pros:

The described protocol is very easy to carry out on a large number of trees per time unit.

b) Cons:

When observed once in a test, it is not always easy to get at the right time to have a good flushing differentiation between the genetic units.

**References**

3.22 Douglas-fir specific protocols

Core group: Jean-Charles Bastien (INRA), Daniel Michaud (FCBA), Jan Kowalczyk (IBL), Mirko Liesebach (vTI now THÜNEN)

3.221 Phenology

Definition
Bud break and bud set are among the most heritable traits of Douglas-fir (Pseudotsuga menziesii [Mirb.] Franco). Provenance tests show a great variability between natural populations for bud flushing date. Interior provenances, which generally originate from high elevation, flush earlier than coastal provenances, whereas in the coastal part of the range, flushing date is mainly driven by the latitude of the geographic origin. Bud break and bud set are very important adaptive traits, related to the tolerance to abnormal climatic events that could occur in the growing environment. Very easy to score on a large number of (young) trees per time unit, these phenology traits are very useful to indirectly assess frost hardiness and, in some extent the stem form (e.g., forking). Genotype’s ranking for bud break and bud set are also very stable across sites and years.

3.221.1 Flushing

Description
Flushing is recorded on each tree separately. The assessment method depends on whether the terminal bud is visible or not.

Scoring scheme
When terminal bud is visible, a score is given to each tree according to the development stage of the terminal bud.

Bud break on terminal bud (5-step score)

<table>
<thead>
<tr>
<th>Scale values</th>
<th>Text</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Dormant bud</td>
</tr>
<tr>
<td>2</td>
<td>Bud enlarges, slightly greenish appearance</td>
</tr>
<tr>
<td>3</td>
<td>Bud scales start to burst; needle points are visible</td>
</tr>
<tr>
<td>4</td>
<td>The needle bundle stretches up to twice the size of the bud size; in general the needles are still tight to the shoot; only rarely are needles around the bud scales splayed out</td>
</tr>
<tr>
<td>5</td>
<td>Needle bundle starts to loosen, shoot still enlarges; the young shoot looks like a paint brush</td>
</tr>
</tbody>
</table>
When the observation is made only once in the growing season, ideal time of recording is when around half of the trees have reached Stage 3. Flushing is better assessed when it is possible to record it repeatedly (e.g., beginning and end of flushing season during a given year or over two different years).

When possible, an alternative (and more accurate) method consists to score 1 or 0 for each tree on a regular time basis (2 to 3 times a week), when the terminal bud reaches Stage 3. This method enables bud break to be expressed in days or degree-days (from a given origin).

When terminal bud is not visible (total height above 3m), a score is given to each tree according to the estimated percentage of crown buds having reached the development Stage 3 described above.

**Bud break on the whole tree (5-step score)**

<table>
<thead>
<tr>
<th>Scale values</th>
<th>Text</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>All buds dormant or just enlarged</td>
</tr>
<tr>
<td>2</td>
<td>1-25 % buds reach Stage 3 or more</td>
</tr>
<tr>
<td>3</td>
<td>26-50 % buds reach Stage 3 or more</td>
</tr>
<tr>
<td>4</td>
<td>51-75 % buds reach Stage 3 or more</td>
</tr>
<tr>
<td>5</td>
<td>76–100 % buds reach Stage 3 or more</td>
</tr>
</tbody>
</table>
Pros and cons of the described protocols

a) Pros:

The protocols described above are very easy to carry out on a large number of trees per time unit (several thousand per day).

They enable a very efficient screening of genotypes for late frost resistance and, indirectly for lowering forking defects.

b) Cons:

For a given trial, bud flushing assessment has to be done in the shortest time possible (ideally within a day).

When observed once in a test, it is not always easy to get the right time to have a good flushing differentiation between the genetic units.

References


3.221.2 Bud set

Description

Bud set assessment enables to indirectly evaluate hardiness to early fall frosts. This trait should be assessed on terminal bud (young trees) only.
Scoring schema
Bud set on terminal bud (5-step score)

<table>
<thead>
<tr>
<th>Scale values</th>
<th>Text</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Terminal shoot is completely green, terminal bud is only allusively differentiated</td>
</tr>
<tr>
<td>2</td>
<td>Terminal bud is very small and visible between the terminal needles; terminal shoot is mainly green, sometimes slightly red</td>
</tr>
<tr>
<td>3</td>
<td>Terminal bud is conical and brown coloration starts; terminal shoot starts to lignify, cork cells are produced</td>
</tr>
<tr>
<td>4</td>
<td>Terminal bud is about 2-3 mm, the brown color is more intense; bark appears mainly grey/greenish</td>
</tr>
<tr>
<td>5</td>
<td>Terminal bud is well developed and dark green; terminal shoot is lignified, that means the bark is greenish-grey, and appear slightly teared</td>
</tr>
</tbody>
</table>

Pro and con of the described protocols

a) Pros:

The above described protocol is easy to carry out on a large number of trees per time unit (several thousand per day).

When possible, it is suggested to assess bud set 2 or 3 times during the growth cessation period in order to better discriminate genotypes.
b) Cons:

Bud set assessment requires a minimum training before starting evaluation on a large number of trees.

This observation is generally more time consuming than flushing; therefore it is highly recommended to assess this trait at nursery stage rather than in the field.

References


3.222 Frost Hardiness

Definition

Frost damage is frequently observed in young plantations. In "frost-prone" sites, damage occurs more frequently than on milder sites and can be severe, leading to mortality, reduced growth and poor stem form. The greatest risk of cold injury occurs in the fall, before shoots are fully hardened for the winter or in the spring when shoots begin, or are about to begin, active growth.

In the field, cold hardiness can be assessed after damaging frost events. However, in order to get a good statistical precision, it is important to ensure that incidence of natural frost injury is not sporadic across field test site and that frost damage can be separated from other causes of injury (e.g., drought, disease, insect). If not, artificial freezing tests on detached shoots should be preferred.

Description

The following protocol is to be applied on a single tree basis.

Subjective 5-step score:

<table>
<thead>
<tr>
<th>Scale values</th>
<th>Text</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Whole plant frozen to death</td>
</tr>
<tr>
<td>2</td>
<td>Terminal shoot frozen and dead, and lateral shoot moderately damaged</td>
</tr>
<tr>
<td>3</td>
<td>Severe damage on lateral shoots only</td>
</tr>
<tr>
<td>4</td>
<td>Little damage on lateral shoots only</td>
</tr>
<tr>
<td>5</td>
<td>No damage visible</td>
</tr>
</tbody>
</table>


Pros and cons of the described protocols

a) Pros:
The above protocol is very fast to implement on a large number of trees in nursery or in field tests.
This evaluation is complementary to bud flushing or bud set assessment to screen genotype's cold tolerance.

b) Cons:
Frost hardiness assessment requires occurrence of a frost event (generally unpredictable)
Frost Damage can sometimes be compounded with disease or insect damage.

References

3.223 Needle cast

Definition
Douglas-fir Swiss needle cast (Phaeocryptopus gaeumannii), is a foliar disease which originates from North America. It induces heavy needle losses, which generally start with the oldest ones. In case of severe attack, only last year’s needles persist. This disease is favoured by a wet climate during the summer season or in wind-protected environments. Damages are observed mainly in young plantations (up to 15 years) and reveal most often mineral alimentation problems (weak growth, shallow soils, etc.). Although less heritable than growth traits, foliage traits appear to be reasonable indicator of Douglas-fir disease tolerance and can help screen for families that show tolerance to Swiss needle cast.
**Description**

The following protocol is to be applied on a single tree basis.

Subjective 5-step score:

<table>
<thead>
<tr>
<th>Scale values</th>
<th>Text</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Very severe infestation (76 % - 100 % needle loss)</td>
</tr>
<tr>
<td>4</td>
<td>Severe infestation (51 % - 75 % needle loss)</td>
</tr>
<tr>
<td>3</td>
<td>Medium infestation (26 % - 50 % needle loss)</td>
</tr>
<tr>
<td>2</td>
<td>Very limited infestation (1 % - 25 % needle loss)</td>
</tr>
<tr>
<td>1</td>
<td>No infestation (0 % needle loss)</td>
</tr>
</tbody>
</table>

**Pros and cons of the described protocols**

a) Pro:

Quick and low cost evaluation method, which can be completed with an evaluation of foliage color (from 1 = yellow o 3 = dark green)

b) Con:

This needle retention evaluation method does not enable to separate tolerance and resistance to Swiss needle cast.

**References**


JOHNSON GR, 2002: Genetic variation in tolerance of Douglas fir to Swiss needle cast as assessed by symptom expression. Silvae Genetica 51 (2/3): 80-86.

3.23 Scots pine specific protocol

Core group: Egbert Beuker (METLA now LUKE),
Jan Kowalczyk (IBL),
Volker Schneck, Mirko Liesebach (vTI now THÜNEN)

3.231 Lophodermium needle cast

Definition
The needle cast disease caused by the fungus *Lophodermium seditiosum* is the most serious foliage disease of Scots pine (*Pinus sylvestris*) in the temperate zone. In some years heavy infections can cause severe defoliation to young pine stands over large areas and a reduction of tree growth. Therefore, the search for resistant or less susceptible populations or individuals is of great significance.

Description of the assessment in general
The assessment of needle cast can easily be recorded at younger plants up to a height of 1 m (average height of a trial series). The whole plant has to be observed. The assessment has to be carried out in spring.

Scoring schema

<table>
<thead>
<tr>
<th>Scale values</th>
<th>Text</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Very severe infection, all needles brown or dropped off</td>
</tr>
<tr>
<td>2</td>
<td>Medium to severe infection, 50-100 % brown needles</td>
</tr>
<tr>
<td>3</td>
<td>Medium infection, 10-50 % brown needles</td>
</tr>
<tr>
<td>4</td>
<td>Weak infection, few brown needles</td>
</tr>
<tr>
<td>5</td>
<td>No infection</td>
</tr>
</tbody>
</table>
1. Very severe infestation, all needles brown or dropped off

2. Medium to severe infestation, 50-100% brown needles

3. Medium infestation, 10-50% brown needles

4. Weak infestation, few brown needles

5. No infection

Photos: S. Ślusarski - IBL

**Pro and con of the described protocols**

The scheme is to easy-to-use. Also the small size of the trees is an advantage for the assessment.

Sometimes it can be difficult to distinguish the damage by *Lophodermium seditiosum* from other sources of browning of needles. For the statistical analysis it also has to be taken into account that the distances between the single values of the scale are not equal. So analysis of variance and estimation of variance components are not possible.
References

3.232 Pine twisting rust

Definition
The pine twisting rust is caused by the fungus Melampsora pinitorqua. It causes severe damage to the shoots of Scots pine seedlings and young trees, resulting in growth reduction, malformation or even death, especially of seedlings. The pine twisting rust is very common in the northern temperate and boreal areas, where also European aspen (Populus tremula L.), which is the alternate host of the pathogen, is common. The pathogen enters the pine seedlings in spring and early summer during shoot elongation. The occurrence of pine twisting rust varies largely between years, due to its strong dependence upon various weather factors. There is large genetic variation in the resistance of Scots pine against the pine twisting rust, both within and between populations.

Description of the assessment in general
Damage by the pine twisting rust can be distinguished most clearly at the end of the summer, when the effected parts of the shoots show resin secretion and become black. The assessments should be made at a young stage, up to a height of about 1 meter, assessing the whole tree. Due to large annual variation in the appearance of the disease, assessments should be made during years when the disease is common or, alternatively, repeated assessments should be made over several years.

Scoring schema
Stem deformation by Melampsora pinitorqua:

<table>
<thead>
<tr>
<th>Scale values</th>
<th>Text</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>present</td>
</tr>
<tr>
<td>1</td>
<td>lack</td>
</tr>
</tbody>
</table>
Pro and con of the described protocols

The scheme is easy to use. Also the small size of the trees is an advantage for the assessment. Especially when not assessed at the right time, the damage may be confused with other shoot deformations, such as those caused by insects.

References


3.24 Mediterranean pines specific protocol

Core group: Eduardo Notivol (INIA),
Allain Bailly (FCBA),
Fulvio Ducci (CRA)

Mediterranean pines are not special from the point of view (need) of protocols standardization. For all generic traits (according to a simple ontology mainly grouped in growth and biomass, phenology, pests and diseases resistance and reproduction), the assessing protocols should be the same that used in others pines or conifers.

The differential focus for this group of species is not “how” but “for what”, and this is because the main purpose of these assessments is related to specific adaptation to the particular Mediterranean environment. When conservation and landscape protection is considered more interesting than wood production the interpretation of the results from measurements are different even when the protocols are the same.

For forest trees, adaptation is important for any kind of environment but when species have to face up to global change, some environments are more susceptible than others to the consequences of that change. In this context there are two groups of traits related with resilience facing drought stress and perturbances, mostly fire.

3.241 Drought

For drought tolerance a good compilation of proxy assessment is included at the end of the document. Drought tolerance is not a trait and it is dependent not only on the plant but on the environment. In addition measuring drought tolerance is not straightforward.

Drought tolerance for the same genotype varies with the soil characteristics, air temperature, water availability, light intensity and their regimes. The interpretation of results from measurements of mortality, soil water content, photosynthetic parameters, transpiration, CO₂ concentration, water potential, water use efficiency assessed by means of isotopic discrimination, phytoregulators concentrations, terpene acids, or other by-products from metabolism, etc. can be used for this purpose but without a standardized protocol due to the different possibilities and framework of testing.

3.242 Flowering

Regarding reproduction, flowering and fructification assessments are not different for the species group. Protocols for cones and flower counting are not different for the Mediterranean pines compared with other species while other traits related to reproduction
like cone serotiny or masting can be highly relevant for Mediterranean pines. Unfortunately there are not agreed protocols for these traits (but see HERNANDEZ-SERRANO, 2014).

Apart from multinomial calibrated scales (0 to 3; 0 to 5 similar to Scots pine or Norway spruce), a continuous variable can be obtained by visually counting strobili (male or female) in a fixed time (for example, 15 seconds), following protocols used in Californian oaks (KOENIG et al. 2009). It has been used with good results in several ongoing papers (HERNANDEZ et al., SANTOS DEL BLANCO et al., CLIMENT et al.). A manual click counter helps homogeneity among observers and observations.

### 3.243 Polycyclism

Polycyclism (the ability for a plant to produce several flushes in the same growing season) can be contemplated as vegetative or reproductive. In Aleppo pine (*Pinus halepensis*), but also occasionally in maritime pine (*Pinus pinaster*), multiple female cone cycles can be observed. Reproductive polycyclism can be recorded as presence / absence in each tree or rate of reproductive polycyclisms compared to a fixed number of reproductive shoots.
Quantitative way of assessment is made by means of counting the number of twigs and number of cycles for rates calculation (PARDOS 2003, GIRARD et al. 2011).

3.244 Bark thickness

Bark can be a paramount trait in protective strategies against forest fires. This trait (bark thickness) is recorded by means of bark calipers with no special requirements. Assessment of this trait is usually made by cross measurements in two directions (N-S and E-W).

References


CLIMENT JM, et al. (in prep): Divergence between subspecies of European black pine (Pinus nigra Arnold) on life history traits.

D2.1 – Common protocols and reference standards for selected traits and species


HERNÁNDEZ, et al. (in prep.): Genetic structure of cone serotiny in Aleppo pine (Pinus halepensis Mill.)


MARTÍN-SANZ R, NOTIVOL E, SANTOS-DEL-BLANCO L, SAN MARTÍN R, CHAMBEL MR, CLIMENT J. Phenotypic plasticity and allometric effects of cone serotiny in a Mediterranean conifer (in prep.)


SANTOS DEL BLANCO et al., (in prep.): Quantitative genetics of male and female reproduction in pines.
3.25 Larch specific protocol

Core group:  **Luc Pâques** (INRA),
            Jan Kowalczyk (IBL),
            Ecaterina Nicoleta Chesnoiu (ICAS),
            Volker Schneck (vTI now THÜNEN)

3.25.1 Phenology

**Definition**

Bud burst and bud flushing are the most critical parts of phenology in larch as they are directly related to the risk of late frost damage. They are also a clear signal of re-start of growth of primary meristems. A large variability at population and individual levels is observed for that trait.

**Way of assessment recommended**

For young trees, that is up to a height the terminal bud is easily observable (2-3 m), observation of bud flushing can be limited to the terminal bud. In this case, at each date of observation, a score is given following the scoring-scale.

Observation can be done weekly if the kinetics of flushing is of interest; if not, it can be done at only one date when 50% of the trees are at Stage 3.
For older and taller trees, that is when total height prevents an easy observation of the terminal bud (above 3 m), the international BBCH scoring system will be favoured and has been adapted to larch. The observation considers all vegetative buds of the whole crown. Scores range from 0 up to 31. Scores 0 to 10 consider the phase up to the first buds reached score 5; scores 11 to 19 evaluate the proportion (from 10 % to 90 %) of buds having reached stage 5; score 31 corresponds to the stage when bud flushed is achieved and first twig elongation is observed. As for young trees, observation can be done weekly or at only one date. Trees are considered flushed at the date when they reached BBCH-score 15.
<table>
<thead>
<tr>
<th>Code BBCH</th>
<th>Stage</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>leafing</td>
<td>All vegetative buds at stage 0 (dormant)</td>
</tr>
<tr>
<td>1</td>
<td>leafing</td>
<td>Most of buds have reached stage 1</td>
</tr>
<tr>
<td>3</td>
<td>leafing</td>
<td>Most of buds have reached stage 2</td>
</tr>
<tr>
<td>7</td>
<td>leafing</td>
<td>Most of buds have reached stage 3-4</td>
</tr>
<tr>
<td>9</td>
<td>leafing</td>
<td>First buds have reached stage 5</td>
</tr>
<tr>
<td>11</td>
<td>leafing</td>
<td>10% of buds with score 5</td>
</tr>
<tr>
<td>12</td>
<td>leafing</td>
<td>20% of buds with score 5</td>
</tr>
<tr>
<td>13</td>
<td>leafing</td>
<td>30% of buds with score 5</td>
</tr>
<tr>
<td>14</td>
<td>leafing</td>
<td>40% of buds with score 5</td>
</tr>
<tr>
<td>15</td>
<td>leafing</td>
<td>50% of buds with score 5</td>
</tr>
<tr>
<td>16</td>
<td>leafing</td>
<td>60% of buds with score 5</td>
</tr>
<tr>
<td>17</td>
<td>leafing</td>
<td>70% of buds with score 5</td>
</tr>
<tr>
<td>18</td>
<td>leafing</td>
<td>80% of buds with score 5</td>
</tr>
<tr>
<td>19</td>
<td>leafing</td>
<td>90% of buds with score 5</td>
</tr>
<tr>
<td>31</td>
<td>growth</td>
<td>Start of twigs elongation</td>
</tr>
</tbody>
</table>

### 3.2.52 Canker

**Definition**

Larch canker due to *Lachnellula willkommii* is the most common disease observed in European larch, especially in alpine populations, but Sudetan and Central Poland populations are not exempt.

Canker produces deformation observable on branches and on stem, including at some stages, resin flow and presence of fructification. In the worse cases, girdling of the stem might be complete.
Method of assessment

Observations can usually start when trees are around 10 years old, rarely before except in case of artificial inoculation.

- Artificial inoculation:
  Inoculation is done through insertion of a calibrated sorgho straw infected by *Lachnellula willkommii*, in a hole drilled by a machine. A nearby hole is performed for control. Young trees (2 years old) and young organs (1-2 years-old branches) can be used (Sylvestre-Guinot and Delatour, 1983).
  Assessment is usually done 6 months after inoculation by measurement of the length (mm) of the canker, the appreciation of the proportion of the twig girdled (ex. 1/3) and by the notation of resin flow and of fructification (presence/absence).

- Observation in nature
  The severity of the disease is appreciated through the presence (1) vs absence (0) of symptoms (0) on stem or branches. The frequency of trees of a given genotype presenting canker is then calculated. (SCHOBER 1985).

Scoring schema

<table>
<thead>
<tr>
<th>Scale values</th>
<th>Text</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Disease present on stem or branches</td>
</tr>
<tr>
<td>0</td>
<td>Disease absence</td>
</tr>
</tbody>
</table>

Method recommended:

Artificial inoculation has the main advantage to allow a much earlier screening of trees and in a relatively fast way (6 months). Compared to observation in natural conditions, the inoculum source is controlled but limited, applied at a high concentration and through man-made damage (hole), which might amplify the response.
Reference


3.253 “Drought” cracks

Definition

Cracks are sometimes visible along larch stem. Their origin is not well known (growth speed, drought?) but is more frequent in drier sites (shallow soil, southern aspect). The defect may be considerable as the cracks usually turn around the stem over one to several meters and can be as deep as the pith. After a few years, the crack might be filled up through healing, which makes the defect less easily observable. But an attentive observation allows it to be detecte through the resulting cicatrix.

All species of larch are susceptible, but more frequently this defect is observed on European larch than on hybrid and finally on Japanese larch.

Way of assessment recommended

The observation requires a close look at stem and to turn around it to have a full vision as the cracks are not necessarily oriented and are usually hidden by branches.

The best season is during winter time when needles have fully fallen off.

The defect seems to appear at a juvenile stage when trees are 10-15 cm diameter thick at BH, which seems a critical stage.
Score

A simple scoring system, namely presence/absence is used.

References

3.26 Conifer non-species specific protocol

Core group (conifer core group leaders):

**Luc Pâques** (INRA; Larch),
Luc Harvengt (FCBA; Norway spruce),
Jean-Charles Bastien (INRA; Douglas-fir),
Egbert Beuker (METLA now LUKE; Scots pine),
Eduardo Notivol (INIA; Mediterranean pines)

3.261 Stem straightness

**Definition**
Stem deformation includes several defects: basal sweep, lining, bending, crookedness, twisting. Their origin can be genetic (due to delayed lignification, production of compression wood?) or accidental. The trunk aspect may change over time with a more or less rapid apparent correction due to the accumulation of growth layers.

![Diagram of stem abnormalities](image)

Lining  Bending  Sinuosity  Basal sweep

It is recommended to separately evaluate these different defects for a better description of stem form and a separation of accidental from genetic effects.

*Stem sinuosity or stem crookedness* (vs *Stem straightness*) aim to assess the external quality of the stem in connection to local deviations from the main tree axis, present along the stem, in any direction. It also includes twisting defects common in some species (larch). Severity of deviations is more or less important: in some cases, the defects disappear externally after a few years, but in the worse cases, they remain over years and are highly detrimental to wood quality. These should be distinguished stem deformation due to intrinsic causes (like due to lignification problems) and stem defects clearly resulting from an...
identified biotic (e.g., *Rhyacionia buoliana* in pines) or abiotic (such as frost/drought damage to terminal buds) factors. In the latter case, a rupture of direction at a given annual increment is observed.

In larch species, stem crookedness is clearly a major defect which might be accidental but which has overall a strong genetic control.

### Description of the assessment

**Age:** For most studies, evaluation should be done when trees are enough developed and better express defects, that is when trees are between (5)-10-15-(20) years old. Afterwards, stem correction through diameter enlargement hides defects which nevertheless remain within the log.

**Season:** Observation should be done during the winter time, when the stem is fully visible, that is in absence of needles.

**Assessment:** it is recommended:

1) to note separately the different defects such as basal sweep, lining, bending and stem sinuosity,

2) to use the scoring scale in an absolute manner (agreed by participants in Leuven) which means that a given score must have the same meaning across different sites;

3) to limit thus observation of crookedness to the part of the stem above 1.30 m (to avoid basal sweep) and avoid the last 2 annual increments: in some species, this part of the stem is heavily influenced by local weather conditions (wind) and corrections of the stem appear in next season(s).

**Before starting observation:**

4) to walk through the plantation and find reference trees for each score, these trees will be marked and referred to several times if needed during the assessment. Picture of these trees should be taken and archived for further documentation.

**During the observation:**

5) to observe trees in two perpendicular planes, so to have a better vision of defects,

6) a given block will be assessed by only one observer, so that observer effects will be included in block effects,

7) in case of doubt between two scores, use the smaller one.

According to available resources, it is recommended to have notation by two independent observers.
Scoring scheme

The following scoring system is proposed: it combines a count of crooks along the stem together with an appreciation of the severity of the crooks.

<table>
<thead>
<tr>
<th>Intensity</th>
<th>none</th>
<th>weak</th>
<th>weak</th>
<th>severe</th>
<th>severe</th>
</tr>
</thead>
</table>

Scores 5 and 4 include what can be seen as ‘straight’ trees: defects in Score 4 are weak enough to suppose that they will disappear with time and have no or little effect on wood quality;

Scores 1 and 2 include ‘crooked’ trees: the defect is so severe that it is supposed to remain over time and strongly affect wood quality (purge needed).

This scoring system can be used in two different ways:

1) in an “absolute” manner, which means that a given score will have the same meaning across different sites;

2) in a “relative” manner: which means to adapt the scoring scale to each site.

Pros and cons

The advantage of the “absolute” manner is that it allows a real comparison of results over sites (and ages); the inconvenience being that in a given site, the distribution of scores will be asymmetric.

The advantage of the “relative” manner is that it allows normalising the distribution of scores; but it prevents an objective comparison among sites. Comparison could only be done through genotype ranking with a loss of information.

At the Leuven workshop (September 2009), participants agreed that the most important factor in international trial evaluation was the possibility to compare genotypes’ behaviour across sites and the absolute way was retained.
References

3.262 Basal sweep

Definition
Basal sweep is a trunk deformation observed at the base of the tree. It can be of genetic origin or accidental (in connection with a poor plantation, poor weed control or wind damage, snow creepage on steep slope). Some species, like maritime pine, larch, etc., are highly susceptible to this defect.

Assessment
A subjective scoring (1/0: presence /absence of the defect) might be enough in most cases. When a more objective evaluation is needed, we recommend the following procedure:

The longer horizontal distance (d) between the deformed tree and a vertical line up to 1.3 m will be measured to evaluate the basal sweep. The vertical line is materialised by a pole standing at the base of the stump and maintained vertical (plumb line, or ). The measure (d) is expressed in centimeters.

Recommendation
The deformation is observed in the plan where the defect is the most severe and up to 1.3 m.

Pros and cons
Several other methods exist as shown in next figure.
### Method 1
- **Advantage**: Most rapid & simple
- **Inconvenient**: But not always 2 points of contact with trunk

### Method 2
- **Advantage**: Adapted to all forms of basal sweep and more precise
- **Inconvenient**: Need a pole with a spirit level and a mobile gauge to measure deviation

### Method 3
- **Advantage**: Apparently convenient method
- **Inconvenient**: - needs 2 measures (150 cm and HT), - difficulties if weed at the base of the tree - difficulties to measure HT with enough precision

**Reference**

### 3.263 Forking

**Definition**
Forking is considered when the main stem axis splits (usually in conifers) into 2 stems of equal importance (similar diameter). Other types of split of the main axis can occur when, for example, a vigorous branch takes an up-right position: in this case, the second axis is usually of a smaller diameter: it is then called ‘ramicorn’.

Forking can be genetically inherited and/or be accidental due to wind, snow, frost, birds, etc., damages. Re-iteration of the defect is possible.

**Assessment**
It is recommended to adopt different protocols according to the age/size of trees:
For young trees (< 15 m), scoring simply identifies trees with or without a fork (1/0).
For older trees (> 15 m), the assessment combines the position of forking occurrence and the number of forks:
### D2.1 – Common protocols and reference standards for selected traits and species

#### 3.2.5 Branching habit

**Definition**

Branching is commonly evaluated through 3 main parameters including branch thickness, branch angle and branch number of primary branches along the trunk. A high density of branches, coarse branches, and branches with an acute angle towards the trunk are indeed considered to downgrade overall stem quality and affect internal wood properties (due to

---

**Table: Forking index**

<table>
<thead>
<tr>
<th>Position: relative to tree height</th>
<th>No fork</th>
<th>Fork</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Upper third</td>
<td>Middle third</td>
</tr>
<tr>
<td>Score relative to position</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Number of axis (forks)</td>
<td>1</td>
<td>n1</td>
</tr>
<tr>
<td>Forking index</td>
<td>= (score x 10)/number of axis</td>
<td></td>
</tr>
</tbody>
</table>

**Example:**

- Number of axes: 1, 2, 2, 2
- Forking index: 40, 15, 10, 5

**Recommendation**

For species with usually no forking re-iteration, the score relative to forking position may be suitable.

For species with usual forking re-iteration, it is recommended to combine both records (position + number) into a forking index: the highest index being for non-forking trees and for trees with a lower number of forks at a higher position.
knot size, angle, density). Thin, flat angle branches at a low density are commonly searched for by breeders.

### 3.264.1 Branch angle

A 4-scale subjective scoring system is used judging the angle made by branches to the main stem: the flatter the angle (close to 90° to the trunk), the better the branching.

<table>
<thead>
<tr>
<th>Scale values</th>
<th>Text</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>branch in the 1\textsuperscript{st} quarter (angle between 67° and 90° angle close to 90°)</td>
</tr>
<tr>
<td>3</td>
<td>branch angle in the 2\textsuperscript{nd} quarter (between 45° and 67°)</td>
</tr>
<tr>
<td>2</td>
<td>branch angle in the 3\textsuperscript{rd} quarter (between 23° and 45°)</td>
</tr>
<tr>
<td>1</td>
<td>branch angle in the 4\textsuperscript{th} quarter (below 23°)</td>
</tr>
</tbody>
</table>

The rationale of this scoring system is that an observer can quite easily figure out the 4 quarters of the angle between the trunk and a perpendicular horizontal line; firstly by dividing the 90° into two parts and then again into two parts. So the observer starts to position the branch in the first (45-90°) or in the second half (0-45°), and then respectively by dividing it in two, in the 1 \textsuperscript{st} or 2 \textsuperscript{nd} quarter or in the 3 \textsuperscript{rd} or 4 \textsuperscript{th} quarter.

**Recommendation:**

Assessment is done at the age of 10 years after plantation, provided total tree height exceeds 6 m. The height (or whorl) at which branching angle is to be assessed will be decided within each trial network.
For some species, at certain ages and sites, branches can hang down at more than 90°. An additional score (5) could be added to take this pattern into consideration.

For some species, branches can start from the trunk at a certain angle and then flatten onwards. Angle from the closest part of the branch to the trunk should be then considered.

### 3.264.2 Branch thickness

A 4-scale subjective scoring system is recommended: it is made more objective by relating primary branch sizes (diameter) to stem diameter.

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>branch diameter less than ( \frac{1}{8} ) stem diameter</td>
</tr>
<tr>
<td>3</td>
<td>branch diameter between ( \frac{1}{3} ) and ( \frac{1}{4} ) stem diameter</td>
</tr>
<tr>
<td>2</td>
<td>branch diameter between ( \frac{1}{2} ) and ( \frac{1}{3} ) stem diameter</td>
</tr>
<tr>
<td>1</td>
<td>branch diameter above ( \frac{1}{2} ) stem diameter</td>
</tr>
</tbody>
</table>

**Recommendation**

Assessment is done at the age of 10 years after planting, provided total tree height exceeds 8 m. In any case, the branches should be easily visible from beneath the trees.

The position of the whorl where branch thickness will be assessed (or the height of branches) will be decided within each trial network.

### 3.264.3 Branch number

The total number of primary branches (above a certain diameter) is an important feature of branching but its consequences on stem and wood quality are also influenced by the way branches are distributed along the trunk.

A two-fold scoring system is suggested combining number and distribution of branches:
Score 1: distribution of branches along the stem
   1 = not in whorls
   2 = in whorls

Score 2: number of branches along the stem or in whorls
   1 = many branches
   2 = moderate number of branches
   3 = few branches

<table>
<thead>
<tr>
<th>Number of branches</th>
<th>Not in whorls</th>
<th>In whorls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Many</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Moderate</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Few</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>

**Comments**
- For some species like larch, branches are not organised in neat whorls so that the distribution score may be useless. In such cases, only the score for branch number will be used.
- If operators are able to identify which branching distribution is more favourable (in relation to wood quality, ...), one could consider combining scores so that the ‘best’ position and low branches numbers give the highest score (i.e., Low number in whorls = 3 x 2 = 6).

**Recommendation**
- The height (or whorl) at which branching number is to be assessed will be decided within each trial network.
- It might be recommended to fix the minimum diameter above which branches are counted.
4. Trait-oriented common protocols

4.1 Wood quality

Manuel Touza Vazquez (CIDTG) (comp.)

There is an increasing interest regarding how to determine wood properties in standing trees with non-destructive techniques (NDTs). This is of particular interest to breeders as these new possibilities may be introduced into breeding programs, allowing, at the same time, the preservation of the original trees.

One of the best known examples is the recent application of acoustic methods for estimating the mechanical properties (modulus of elasticity) in standing trees.

Other available technologies include penetrometers (pylodin), drilling resistance methods (resistograph), measurement of longitudinal surface growth strains with strain gauge devices (as the one developed by CIRAD-Forêt), etc.

In other cases, traditional lab techniques together with X-Ray densitometry, fractometers, etc., allow the determination of an increasing number of wood properties (density, sapwood-heartwood ratio, early-wood-late-wood ratio, bending strength, compression strength...) in increment cores.

NIR techniques also allow the determination of an increasing number of wood properties from wood cores and/or wood power.

Due to the previous reasons, a questionnaire was drawn up on non-destructive methods for assessing wood properties in standing trees and distributed by CIDTG in September 2014 to WP2 and WP11 partners.

The questionnaire was based on the outcome of a previous survey carried out in the frame of WP11 and was structured into four parts (General overview of available techniques and equipment; Acoustic tools; Near infrared Spectroscopy and Innovation, and future trends).

The objective of the survey was to gain information on existing equipment and protocols for determining wood properties in standing trees as well as to identify gaps and future trends.

For the purpose of the questionnaire, only wood properties determined on standing trees or small wood samples (as a wood core or wood power) may be considered as non-destructive as they will allow the preservation of the individual tree.

Twelve partners from ten countries responded to the WP2 survey (Partners 1-INRA [FR], 3-ALTERRA [NL], 6-BOKU [AT], 8-CIDGT [PO], 9-CNR [], 10-CRA [], 12-FCBA [FR], 18-IICT [], 20-INNVENTIA [], 24-METLA (now LUKE) [FI], 26-UGent [BE] and 28-vTI (now THÜNEN) [DE]).

The survey was structured into four parts:
Part I. General overview of available techniques and equipment
Part II. Acoustic tools
Part III. NIR
Part IV. Innovation and future trends
The responses were analysed in October 2014 and presented at the 3rd Annual meeting in Tulln (AT). The main results are discussed below.

### 4.11 General overview of available techniques and equipment

A map was created of the available techniques that are employed and/or being evaluated for determining wood properties in standing trees. At the same time, several institutions are evaluating the future use of the same techniques.

<table>
<thead>
<tr>
<th>Project partners from ten countries responded to the WP2 survey</th>
</tr>
</thead>
<tbody>
<tr>
<td>BOKU</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>Pylodin</td>
</tr>
<tr>
<td>Resistograph</td>
</tr>
<tr>
<td>Acoustic methods</td>
</tr>
<tr>
<td>Wood cores Trad. Lab.</td>
</tr>
<tr>
<td>Wood cores X-Ray densit.</td>
</tr>
<tr>
<td>Wood cores NIR</td>
</tr>
<tr>
<td>Wood cores Colorimeter</td>
</tr>
<tr>
<td>Rigidimeter for MoE</td>
</tr>
</tbody>
</table>

*now THÜNEN

**Codes employed:**
- **Green**: Employing the technique
- **Yellow**: Evaluating the future use
- **Orange**: Most experience in other fields (timber construction)

Only four technique, comprise the 80 % of the total that are being employed for determining wood properties in standing trees.
Wood density in standing trees is mainly determined employing a wood core. Traditional volumetric methods and X-Ray densitometry are being employed in similar percentages.

The next graph points out how the different techniques agree with the expectations of the users (1 Absolutely agree, 0.6 Rather agree, 0.3 Rather disagree, 0 Absolutely disagree).
Half of the institutions that answered the survey are evaluating new techniques for determining wood properties in standing trees. NIR, resistograph and acoustic methods comprise 80% of the techniques being evaluated.

4.12 Acoustic tools

A map of the equipment being used, as well as the species tested.

<table>
<thead>
<tr>
<th>Equipment</th>
<th>CIDGT</th>
<th>FCBA</th>
<th>INNVENTIA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Director ST 300</td>
<td>x</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Microsecond Timer</td>
<td>x</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IML Hammer</td>
<td>x</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hitman</td>
<td></td>
<td></td>
<td>(x)</td>
</tr>
<tr>
<td>Ultrasonic Timer</td>
<td>x</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Species tested</th>
<th>CIDGT</th>
<th>FCBA</th>
<th>INNVENTIA</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pinus pinaster</em></td>
<td>x</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td><em>Picea abies</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Pinus taeda</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Pseudotsuga menziesii</em></td>
<td>x</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Eucalyptus spp</em></td>
<td></td>
<td></td>
<td>(x)</td>
</tr>
<tr>
<td><em>Populus spp</em></td>
<td></td>
<td></td>
<td>(x)</td>
</tr>
</tbody>
</table>
4.13 Near Infrared Spectroscopy (NIR)

Six of the twelve partners that answered the questionnaire are using NIR and two others are planning to do in the future.

<table>
<thead>
<tr>
<th></th>
<th>BOKU</th>
<th>FCBA</th>
<th>IICT</th>
<th>INNVENTIA</th>
<th>CNR</th>
<th>INRA</th>
<th>METLA (LUKE)</th>
<th>U Gent</th>
</tr>
</thead>
<tbody>
<tr>
<td>More than ten years</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between 5-10 years</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>x</td>
<td>x</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Planning to do so in the future</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>x</td>
<td>x</td>
</tr>
</tbody>
</table>

Together with traditional properties such as chemical composition or density, NIR is being used increasingly in new applications such as wood identification, durability, surface weathering, etc.

The partners were asked to select whether or not the following statements hold true for them (1 Absolutely agree, 0.66 Rather agree, 0.33 Rather disagree, 0 Absolutely disagree).

<table>
<thead>
<tr>
<th>Statement</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>NIR may save money and time in the determination of wood properties</td>
<td>0.94</td>
</tr>
<tr>
<td>It is better to work with wood cores for having an idea of the radial variation of the property</td>
<td>0.94</td>
</tr>
<tr>
<td>NIR will have an increasing use in the forestry sector</td>
<td>0.83</td>
</tr>
<tr>
<td>It is better to work in the laboratory with a properly seasoned sample than try to determine a quick reference value in the field</td>
<td>0.78</td>
</tr>
<tr>
<td>NIR seem the most promising technique for determining new wood properties in standing trees</td>
<td>0.66</td>
</tr>
<tr>
<td>NIR equipment is expensive and require a well-trained, staff so its use will be restricted to a reduced number of research institutions</td>
<td>0.61</td>
</tr>
</tbody>
</table>
The answers pointed out a clear agreement that NIR may save money and time in the determination of wood properties, and as well that it is better to work with wood cores to obtain an idea of the radial variation of the property.

### 4.14 Innovation and future trends

The next graph points out the main interests in employing NDTs in standing trees (1 Absolutely agree, 0.6 Rather agree, 0.3 Rather disagree, 0 Absolutely disagree).

All the partners absolutely agree that their main interest in using NTDs it a better understanding of the effect of silviculture on wood properties as well as to improving the traditional breeding programs introducing new criteria.

The next graph shows the future trends regarding the use of NDTs in standing trees (1 Absolutely agree, 0.6 Rather agree, 0.3 Rather disagree, 0 Absolutely disagree).

There is a clear agreement that cooperation and the definition of common protocols are essential for the development of NTD’s.
The following is a list of innovative methods that have been recently developed by the partners for the assessment of wood properties in standing trees:

- CIDGT: Tension wood in standing *Eucalyptus globulus*
- CIDGT: Acoustic methods for determining MoE in young trees (work in progress)
- CNR-IVALSA: X-ray tomography (density distribution and 3D maps)
- CNR IVALSA: Hyperspectral imaging (variation of wood properties in space, spectral maps)
- IICT: Physical and chemical characterization using core sampling
- INNVENTIA: Radial variations in spiral grain for some hardwood species (work in progress with larch)
- INRA: Rigidimeter on larch and Douglas fir
- U GENT: Microdensitometrical analysis of long wood cores with micro-CT

The partners were asked if they were interested in testing any particular wood property that cannot be predicted nowadays. The following table shows the answers.

<table>
<thead>
<tr>
<th>Property</th>
<th>BOKU</th>
<th>CNR IVALSA</th>
<th>FCBA</th>
<th>IICT</th>
<th>INNVENTIA</th>
<th>INRA</th>
<th>METLA (LUKE)</th>
<th>vTI (THÜNEN)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vulnerability to drought stress</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Complete characterization of material – multisensory approach</td>
<td></td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Retrospective assessment of annual growth</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>x</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydraulic conductivity using small branches in <em>Pinus pinaster</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>x</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole-timber wood homogeneity and performance during wood drying</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chemical composition and heat value</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>x</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spiral grain, Heartwood/sapwood limits</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>x</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NIR models for the constitutive stilbene content in Scots pine heartwood or induced content in other tissues</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>x</td>
</tr>
</tbody>
</table>
References


HANSEN C. P. 2002: Application of the Pilodyn in Forest Tree Improvement. Danida Forest Seed Centre. (Technical Note no. 55).


D2.1 – Common protocols and reference standards for selected traits and species


4.2 Drought resistance

Maria Cristina Monteverdi (CRA) (comp.)

Introduction
The report of the IPCC in 2007 confirms that in the last decades the climate is changing, significantly affecting the natural resources on Earth. Several studies have shown how climate change may affect the thermal regimes and precipitation, they are changing very important physiological processes of ecosystems on our planet. According to the IPCC report (2007), although the effect space-time climate change is still partially uncertain, it seems certain that the adaptability / resilience of ecosystems, including forests, is at serious risk. Hence the importance of studying the adaptive biodiversity in relation to climate change at the inter - intraspecific of forest species, in order to identify adaptive traits useful for the sustainable management of genetic resources. Most climate predictive models show increased temperature trends of, and decreased precipitation trends with, a consequent increase of evaporation rate. So the drought stress is one of important abiotic factor that affect natural ecosystems, also forest ecosystems. Therefore, to assess the adaptive traits at inter – intraspecific levels, they play an important role to identify early selection parameters, suitable for new breeding programs of forest reproductive material, able to tolerate the drought condition maintaining a good productivity. All of these tools help to produce forest reproductive material suitable for a sustainable forest ecosystem management.

The purpose of this work is to find out a reference method for a joint evaluation that could be identified and shared by scientific community. In particular, the purpose is to obtain information on existing methods to assess drought resistance on their quantitative, qualitative, and operational significance and to identify eventual gaps. A “Survey on different methods to assess drought resistance and tolerance and related adaptive traits” was created and sent to participants:

Some authors defined (LARCHER 1980, TESCHE, 1992) the drought resistance, such as the ability of a plant to ensure its life processes, as keeping its water balance positive during a period of drought as long as possible. In the following, the term of drought resistance will be used as a generic term to describe the ability of tree plants to tolerate, resist, and avoid different levels of water stress.
STRESS RESISTANCE (LEVITT 1972)

**Stress avoidance**

Stress avoidance/escape (ability to avoid stress)

These are characters incorporated into the genotype, but they are not really resistant plants.

Examples:
- Ability of some plants to lose their leaves in the case of water stress
- Effimer plants of the desert who make the life cycle only when it rains.

**Tolerance (real resistance to stress)**

Avoidance of strain to stress

For example, Drought stress - Es: maintaining cell turgor through osmotic adjustments or cell wall elasticity.

Tolerance of strain caused by stress

Sample: desert plants that reach extremely low values of water potential and continue the work (the presence of a little water in the tissues, but the ability to maintain active metabolism with internal intrinsic mechanisms. For example, greater quantity of water bound to the plasma membrane.)

### 4.21 Methodology and Evaluation System

The survey is structured into three main parts:

A. Overview of parameters, methods, and species investigated.
B. Past experiences in drought resistance and tolerance studies (participation to projects, and scientific papers).
C. Evaluation of methods according to the following criteria:
   - objectiveness;
   - capacity building;
   - simplicity;
   - destructiveness;
   - cost.

### 4.22 Preliminary results

**Participation of involved Institutions**

Until now, ten Institutions from nine countries answered the questionnaire on a total of 20 participant Institutions on WP2+WP3+Wp11 (Table 4.2.1). Only 10 of 20 participant institutions answered the questionnaire, of these only 4 of 10 are studying or studied drought resistance.
Table 4.2.1: Participating institutions involved with the questionnaire, the number of those interested and those that have not answered.

<table>
<thead>
<tr>
<th>N. participant institutions (WP2+WP3+WP11)</th>
<th>N. respondent institutions</th>
<th>N. Institutions studying drought resistance</th>
<th>N. Institutions not studying drought resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>10</td>
<td>4</td>
<td>6</td>
</tr>
</tbody>
</table>

Figure 4.2.2: Results of participation

The geographic distribution of the institutions that answered the questionnaire shows two distinct groups: those involved in the assessment of drought resistance (Mediterranean countries), and those not involved in the study of drought resistance (northern Europe countries).
Figure 4.2.3: Geographical distribution of participant institutions that answered to drought resistance questionnaire. The red color indicates the countries involved on drought resistance studies, the blue ones the countries currently not involved on drought resistance studies.

Table 4.2.2: Countries, institutions, and drought resistance interest

<table>
<thead>
<tr>
<th>Countries</th>
<th>Respondent institutions</th>
<th>Drought resistance interest</th>
</tr>
</thead>
<tbody>
<tr>
<td>Austria</td>
<td>BOKU - Vienna</td>
<td>Yes</td>
</tr>
<tr>
<td>Netherlands</td>
<td>ALTEGRA - Wageningen</td>
<td>Yes</td>
</tr>
<tr>
<td>Spain</td>
<td>INIA ACoruna</td>
<td>Yes</td>
</tr>
<tr>
<td>Spain</td>
<td>CITA - Aragon</td>
<td>Yes</td>
</tr>
<tr>
<td>Italy</td>
<td>CRA SEL</td>
<td>Yes</td>
</tr>
<tr>
<td>UK</td>
<td></td>
<td>No</td>
</tr>
<tr>
<td>Germany</td>
<td>vTI (now THÜNEN)</td>
<td>No</td>
</tr>
<tr>
<td>France</td>
<td>FCBA</td>
<td>No</td>
</tr>
<tr>
<td>Finland</td>
<td>METLA (now LUKE)</td>
<td>No</td>
</tr>
<tr>
<td>Belgium</td>
<td>VLAGEW</td>
<td>No</td>
</tr>
</tbody>
</table>

Overview of parameters, methods, and species investigated

From the questionnaire, it appears that the forest species of interest about the drought resistance are the following: Scots pines, black pines, Mediterranean pines, Norway spruce, aspen, beech, ash, Pubescent oak, rowan, maple, oaks, Douglas fir, wild cherry, and silver fir.

Table 4.2.3 shows the main objectives of the study declared by involved institutions, with the relative parameters investigated, forest species, and methods used.
## D2.1 – Common protocols and reference standards for selected traits and species

### Objectives

<table>
<thead>
<tr>
<th>Parameters studied</th>
<th>Species investigated</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Evaluation of drought effects on biomass production</td>
<td>Air moisture, soil moisture, physiological drought</td>
<td>Norway spruce</td>
</tr>
<tr>
<td>Individual survival</td>
<td>Scots pine, back pine, Mediterranean pines</td>
<td>Watering restriction in nursery /greenhouse /phytotron, weighing method, TDR probes</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bench top dehydration, air injection, acoustic emission testing</td>
</tr>
<tr>
<td>Dry weight ration (leaf, stem, root)</td>
<td>Oven and balance (dry at 70 °C for 72 h)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mass spectrometer to measure carbon isotopic composition (δ(^{13})C)</td>
<td></td>
</tr>
<tr>
<td>Photosynthetic parameters (A, g(_\text{c}), T)</td>
<td>Gas exchange analysis, portable gas exchange analyser (CIRAS-2, PPSystem)</td>
<td></td>
</tr>
<tr>
<td>Chlorophyll fluorescence of photosystem II (PSII)</td>
<td>Portable fluorescence monitoring system (Hansateck Instruments, FMS 2)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HPLC (High Pressure Liquid Chromatograph)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Enzyme extraction and spectrophotometric assay</td>
<td></td>
</tr>
<tr>
<td>Photosynthetic pigments concentration (Chl(_a), Chl(_b), xanthophylls cycle)</td>
<td>Proline extraction and determination (Bates et al. 1973) spectrophotometer</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Liquid chromatography coupled to tandem mass spectrometry (Durbanse et al. 2005)</td>
<td></td>
</tr>
<tr>
<td>Antioxidant enzyme activities</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proline production</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phytohormone production</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Xylem anatomy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Root morphology</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydraulic architecture</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P(_{50}) (the applied air pressure causing 50 % loss of conductivity)</td>
<td>Cavitron technique (for cavitation vulnerability curves (Cochran et al. 2005))</td>
<td></td>
</tr>
<tr>
<td>Water relations at cell level</td>
<td>Free transpiration method and sap expression methods, Scholander pressure chamber</td>
<td></td>
</tr>
<tr>
<td>Phenological plasticity</td>
<td>Scoring system, phenological camera (Twingscapes TimelapseCam)</td>
<td></td>
</tr>
<tr>
<td>Cambial phenoology</td>
<td>Microscopic method</td>
<td></td>
</tr>
<tr>
<td>Carbon isotope discrimination δ(^{13})C (structural carbon and soluble sugars), WUE</td>
<td>Extraction methods (Brugnoli et al. 1988) measures carbon isotopic composition (δ(^{13})C), dendro-isotopic analysis</td>
<td></td>
</tr>
</tbody>
</table>

### To study the adaptation to climate change

<table>
<thead>
<tr>
<th>Parameters studied</th>
<th>Species investigated</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water Use Efficiency (WUE)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Photosynthetic parameters (A, g(_\text{c}), T)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chlorophyll fluorescence of photosystem II (PSII)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fv/Fm ratio, (\phi_{\text{PSII}}) (quantum yield of PSII photochemistry), (q_{\text{P}}) (photochemical quenching), NPQ (non photochemical quenching)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Photosynthetic pigments concentration (Chl(_a), Chl(_b), xanthophylls cycle)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antioxidant enzyme activities</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proline production</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phytohormone production</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Xylem anatomy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Root morphology</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydraulic architecture</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P(_{50}) (the applied air pressure causing 50 % loss of conductivity)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water relations at cell level</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phenological plasticity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cambial phenoology</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carbon isotope discrimination δ(^{13})C (structural carbon and soluble sugars), WUE</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4.2.3: Main objectives of the study declared by involved institutions, with the relative parameters investigated, forest species, and methods used

It can be noted overall, that most of the parameters used for the assessment of drought resistance are physiological parameters (64 %), while the quantitative, morphological and phenological parameters are used less, 14 % to 14 % - 9 % respectively.
Past experiences in drought resistance and tolerance studies (participation to projects, and scientific papers)

The analysis of the results obtained from the questionnaire, indicates that of the institutions that declared they work or have worked on drought resistance, 3 out of 5 have experience in drought resistance and tolerance measurements prior to the year 2000, while 3 out of 5 started studies on drought resistance between 2000 and 2005.

Four institutions out of 20 are still studying drought resistance aspects, 1 out of 20 stopped the scientific activities on drought resistance, while 5 out of 20 not actually involved in drought resistance assessments, and more than 11 out of 20 did not answer the questionnaire (Figure 4.2.4).

Most of the institutions are involved in international projects and networks, while only a small part is only involved in national programs and projects on drought resistance assessment.

Most of the institutions said they don't have specialized staff. About half of institutions have acquired the technical/scientific knowledge on drought resistance by learning by themselves, while a smaller part conducted postgraduate and undergraduate studies, specialized commercial courses in other countries in related subjects.

Methods evaluation

Objectiveness of methods

The partners involved in the questionnaire indicated 21 different methods for assessing drought resistance. All methods are described in the literature, and almost all are standardized methods according to existing protocols.
Capacity building

Only 3 out of 21 methods below are easily applicable in the field. Approximately half of below methods require voluminous equipment, and only a few (2 out of 21) need for special infrastructure. Almost all of the below methods (86%) allow statistically valid information to be acquired in a short time. On 21 methods about half allow measurements of multiple parameters to be collected at the same time.

Destructiveness

Most of the methods identified are not destructive.

Table 4.2.4: Estimation of destructiveness and/or invasiveness of methods

<table>
<thead>
<tr>
<th>Method</th>
<th>Invasive</th>
<th>Destructive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bench top dehydration</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Incremental reaction to reduce air moisture</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Watering restriction in nursery/greenhouse/phytotron</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Dry weight ratio</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Stable isotope analysis</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Photosynthetic pigments analysis (Chl a; Chl b; xanthophylis cycle)</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Antioxidant enzymes activities</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Proline and phytohormone production</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Xylem anatomy</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Root morphology</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Hydraulic architecture</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Pressure-volume curves</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Cavitation curves</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Gas exchange method</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Scoring system, phonological camera</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Cambial activity / microscopic method</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Extraction of soluble sugars methods (BRUGNOLI et al. 1988), measures carbon isotopic composition (δ¹³C), dendro-isotopic anylysis</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

Costs

The cost analysis is currently in progress due to the absence of direct information.

4.23 Conclusions

Currently we can only deduce that the physiological traits are the most investigated to assess the drought resistance. This is probably because of the complexity of information that can be provided, but also for the capacity building of the methods used for their assessment. Normal are methods that allow statistically valid information to be acquired in a short time.
and are often able to provide simultaneous measurement of multiple parameters. Moreover they are generally not destructive methods.

In any case, due to the low number of questionnaires filled out, the results obtained until now do not allow a reference method for a joint evaluation to be identified and shared by scientific community. So these results cannot be considered definitive. In order to get a valid result, the questionnaire should also be sent to the remainder of the project participants and integrated all with the results obtained in TREEBREEDEX project. Otherwise, it could be a good solution to take as reference the results obtained in the TREEBREEDEX project about the reference method for drought resistance assessments.

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**D2.1 – Common protocols and reference standards for selected traits and species**


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5. **Reference genotypes**

Luc Pâques (INRA) (comp.)

**Definition**

In the course of the TREEBREEDEX project, the need to share common reference genotypes for main forest tree species was noted several times. This might be useful for several research purposes.

The concept “genotypes” encompasses different types of genetic units: seed stands, seed orchards, full-sib progenies, half-sib progenies and clones. According to research needs, at least three different types of reference genotypes have been identified, directly linked to genetics & breeding activities or more broadly to ecological studies.

**Type 1 ‘control genotypes’**: in the course of their breeding programme, breeders evaluate their own genotypes and candidate new varieties (for example for certification) relative to some controls towards which they rank their genotypes and estimate genetic gains. Comparison of gains is rarely possible at international level because of this lack of common references.

**Type 2 ‘signal genotypes’**: evaluation of some traits is complex because either it relies on subjective scoring (e.g., stem straightness, branching) or it is done relative to known genotypes within a restricted collection of genotypes available in a country (e.g. phenology, disease resistance). ‘Signal’ genotypes would be useful as references along scoring scales (straight vs crooked/ early flushing vs late flushing/ resistant vs sensitive, etc). They would be unavoidable for international evaluation and later on for comparison of results.

**Type 3 ‘widespread common genotypes’**: for some ecological studies, in particular to monitor climate changes impact (phenotypic plasticity study; phenology modelling; pest & disease epidemics monitoring; etc), the creation of a dense grid of sites with the same genotypes, across contrasted ecological conditions through Europe, would be highly beneficial. To be feasible, the creation of this network should be done in a soft, low-cost way: for example in any new field trial, some few trees (how many?) from various genotypes of some species could be systematically planted, additionally to the main experiment.

Further description will be given below. To be successful, conditions are that:

- a) we are able to identify such genotypes for a given set of species of commercial interest,
- b) we are able to make them available to the scientific community,
- c) we agree to use them as much systematically as possible.

**Objective**

The objective of this task is to come up with a roadmap to build up this “reference genotype” provider service and hopefully to implement it as far as we can for a couple of species.

Steps include:
identification at national levels of existing genotypes with adequate properties and/or of possibilities to identify such genotypes: collection of this information, agreement on a list of suitable ‘reference genotypes’,

identification of national (infra-)structures which could potentially offer this ‘sharing’ service (seed storage facilities, veg. prop. facilities (nurseries, tissue culture/ somatic embryogenesis lab, etc.)):: these structures would have the responsibility to store/preserve the material (seed storage, cryoconservation, rootstock hedges...), to mass-produce it (collect seed lot or buy it, veg. prop. clones) and to disseminate it according to needs. Most probably more than one structure/species is wished.

identification of technical, legal and financial conditions to reliably establish such service.

**Description of reference genotypes**

The nature, the characteristics and the use of these reference genotypes will vary according to their objective.

<table>
<thead>
<tr>
<th></th>
<th>Type1: ‘Control’</th>
<th>Type2: ‘Signal’</th>
<th>Type3: ‘Widespread’</th>
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</thead>
</table>
| Nature of genotypes | Variable according to the species breeding strategy and its level of advancement:  
- synthetic varieties from seed orchard (ex. for conifers)  
- clones (ex. for veg-prop. based strategy: ex. poplar, wild cherry)  
- selected seed stands (i.e., starting programme) | Preferably clones  
But some other material (FS...) might be necessary when veg. prop. is not/hardly possible | Preferably clones  
But some other material might be necessary when veg. prop. is not/hardly possible (FS progenies...?) |
| Characteristics   | Genotypes of a given species, the best known material  
‘Regionalised’ (according to biogeographic zones) material could be needed according to species (ex. Norway spruce) | Genotypes of a given species; contrasting pairs of genotypes for given set of traits representing extreme scores on evaluation scale  
‘Regionalised’ material could be needed according to species | Genotypes from several species; from different bioclimates across Europe |
| Use               | In international and national genetic trials (e.g. evaluation, certification trials) | In international and national genetic trials of all types | Potentially in all new (genetic) trials to be established, whatever the species tested |
| Conditions        | - mandatory in international trials  
- free but recommended in | - mandatory in international trials  
- free but recommended in | Recommended |
### Volume of plants

<table>
<thead>
<tr>
<th>National trials</th>
<th>National trials</th>
<th>National trials</th>
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<tbody>
<tr>
<td>Like any other genetic units tested in the trial for a given species</td>
<td>A few (2-5?) ramets / clone or sibs / family (&lt;10?) for a given species</td>
<td>A few (5-10?) trees from each species, whatever the main species tested</td>
</tr>
</tbody>
</table>

**Remarks:**

Whatever the types of reference genotypes,

1) Genotypes should be (as) stable (as possible) in their genetic composition (collection only in good flowering years, mixture of crops from several yrs, ...) and their genetic conformity guaranteed. Reference DNA samples could be stored at the Repository Centre (AIT) for control.

2) The most straightforward references to implement this service will be genotypes already mass-produced (from seed orchards, commercially propagated clones), certified and commercially available: these should be probably favoured whenever they fit the above requirements (like for type 1 and perhaps type 3 genotypes above).