



Treebreedex

TREEBREEDEX

RESEARCH INFRASTRUCTURE NETWORK 2006 - 2011
(European project CT2006-026076)



REFERENCE PROTOCOLS FOR ASSESSMENT OF TRAITS
AND
REFERENCE GENOTYPES TO BE USED AS STANDARDS
IN INTERNATIONAL RESEARCH PROJECTS



CRA - SEL Consiglio per la Ricerca e la sperimentazione in Agricoltura
Centro di Ricerca per la Selvicoltura - Arezzo
Forest Genetic Resource Laboratory



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Compiled by

*Fulvio Ducci (CRA SEL, IT), Bart De Cuyper (INBO, BE), Roberta Proietti (CRA SEL, IT),
Luc E. Pâques (INRA, FR), Heino Wolf (SBS, DE)*



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2012

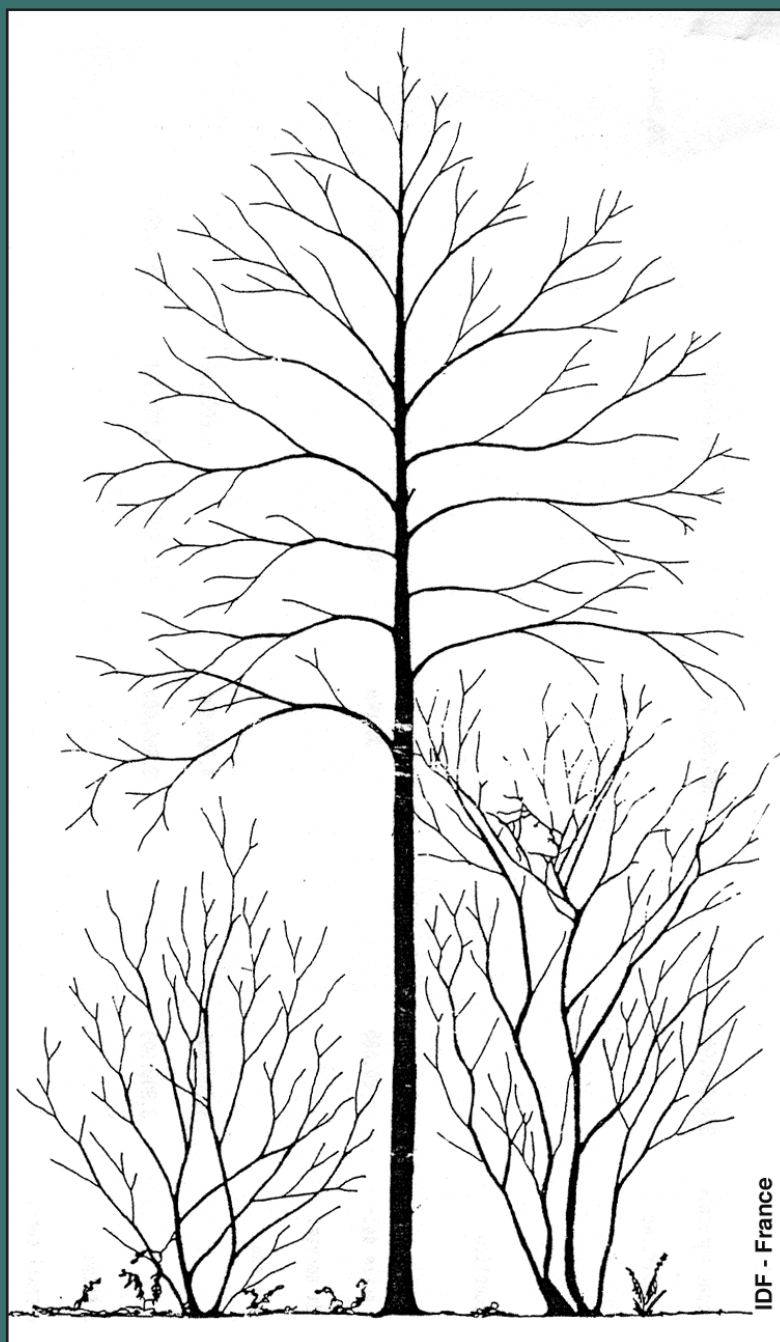


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(European project CT2006-026076)

MORPHOLOGICAL TRAITS



IDF - France

Compilers: Fulvio Ducci (CRA SEL, IT), Bart De Cuyper (INBO, BE), Luc E. Pâques (INRA, FR), Roberta Proietti (CRA SEL, IT), Heino Wolf (SBS, DE).

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FOREWORD

The protocols presented here are proposed as **reference protocols** to be used in international evaluation of common trials. Their aim is not to interfere with local habits and ways of doing which might be preferred for domestic use. For international evaluation, it is necessary that partners speak a “same language” and have common references to describe and evaluate traits: this will facilitate joined analysis of datasets, allow precise comparisons among sites and a more correct interpretation of results. In addition, it should progressively allow building up a better unified *corpus* of genetic parameters among the scientific community and thereby it will help getting a clearer picture of genetic parameters trends within species. Comparisons among published international results are currently hardly possible.

Reference genotypes are proposed as well for some species. They can be used as standards across international field experiments and facilitate the interpretation of results from site to site. They usually represent extreme phenotypes for a given trait. They will be used as ‘signal’ trees like for phenological traits or as scoring-scale references or standards to which datasets should be adjusted. These references should be proven stable genotypes marked by a low $G \times E$ interaction and should be easily mass-produced so to be shared among participants. Protocols are mostly proposed for traits commonly assessed by breeders and for which an agreement can probably be easily found, but they include also some original protocols developed in a given team for a specific trait and scientific question: these latter protocols are not subject to consensus but they are worth to be known and shared and they can serve as well as reference protocols or at least as a starting point for further protocol development.

The list of reference protocols is not exhaustive and should be enriched over time.

Luc E. Pâques

Coordinator

February 23, 2011

CONTENTS

GENERAL REMARKS

MORPHOLOGICAL TRAITS

Stem straightness	Conifers
Basal sweep	Conifers/broadleaves
Stem straightness	Broadleaves
Branch angle	Conifers/broadleaves
Branch thickness	Conifers/broadleaves
Apical dominance	Conifers/ Broadleaves
Forking	Conifers/broadleaves

ADAPTIVE TRAITS

Protocol for assessment of phenology traits	Definitions – recommendations
Bud break	<i>Abies</i> spp.
Bud burst	<i>Larix</i> spp.
Bud burst, bud break, termination of growth	<i>Picea</i> spp.
Bud break, shoot elongation	<i>Pinus sylvestris</i>
Bud break, Bud set	<i>Pseudotsuga menziesii</i>
Bud break, bud set, termination of growth	<i>Populus</i> spp.
Bud break	<i>Prunus avium</i>
Leaf senescence	<i>Larix</i> spp.
Flowering phenology (female and male)	<i>Abies</i> spp.
Flower phenology (female and male)	<i>Pinus sylvestris</i>
Flower phenology (female and male)	<i>Populus</i> spp.
Flowering and fruit phenology	<i>Prunus avium</i>

ADAPTIVE TRAITS: OTHER TRAITS

Assessment of frost hardiness	Conifer/broadleaves
Biomass assessment	Conifer/broadleaves

GENERAL REMARKS

- In case of a block design, each block should be assessed by only one observer/staff, so that observer effects will be included in block effects;
- in case of a trial with a completely randomized design (single tree plots) is assessed by several observers, at least 50 trees should be assessed by each one of them so to fine-tune the scoring system and to assure a consensus on the interpretation of the scoring system;
- before starting the observation it is recommended to walk through the plantation and find out reference trees for each score class. These trees will be marked and referred to several times if needed during the assessment. Pictures of these trees should be taken and archived for further documentation;
- in case of doubt between 2 scores, intermediate scores (e.g. 3.5) are allowed;
- scoring scales should be used in an absolute manner which means that a given score must have the same meaning across different sites, thus allowing the comparison of genotype behaviour in international and/or multi-site experiments.

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.

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

The advantage of the “relative” manner is that it allows normalizing 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.

In Leuven Treebreedex workshop (09/2009), participants agreed that the most important in international trial evaluation was the possibility to compare genotypes behavior across sites and the absolute way was retained.

Morphological trait: **Stem straightness**

Conifers

Date: March 2010

PROTOCOL FOR ASSESSMENT OF STEM STRAIGHTNESS IN CONIFERS

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.

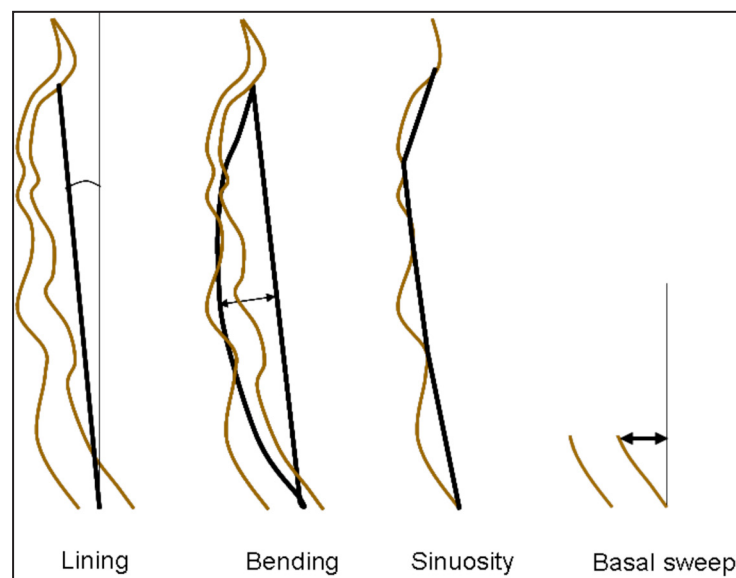


Figure 1. Definition of stem defects in Conifers.

It is suggested to evaluate separately these different defects for a better description of stem form and a separation of accidental from genetic effects (Figure 1).

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 directions. It includes also twisting defects common in some species (larch). Severity of deviations is more or less important: in some cases, the defects externally disappear after a few years but in the worse cases, they remain over years and are highly detrimental to wood quality. Should be distinguished stem deformation due to intrinsic causes (like due to lignification problems) and stem defects clearly resulting from identified biotic (e.g. *Rhyacionia buoliana* in pines) or abiotic (as frost/drought damages to terminal buds) factors. In the latter case, a rupture of direction at a given annual increment is observed.

ASSESSMENT METHODS

Objective assessment methods have been proposed by several authors to evaluate stem form (Contrain, 1993; Campbell, 1965; Shelbourne and Namkoong, 1966; Powell, 1987). Most of them rely on measurement of deviations from pictures taken in two perpendicular plans or from direct measurements in the field (Contrain, 1993). Unfortunately, these methods are time-consuming and are only worth for specific studies on a limited number of trees.

For routine assessment, a subjective scoring system is preferred because it is fast and applicable on large sets of

trees as in tree breeding. It describes a continuum from straight stems to severely crooked trees. Proposed scales run from 1 to 3 up to 1 to 9. Scoring systems referring strictly to stem straightness/stem crookedness are preferred to those confounding the different stem form defects.

A two small scale span (like 1 to 3) is convenient but it suffers from a lack of precision; a too wide scale (over 1-9) is more delicate to apply and the separation between two scores becomes more subjective. It is then proposed to have an intermediate scale (1-5/1-6).

SCORING SYSTEM

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 (Figure 2).

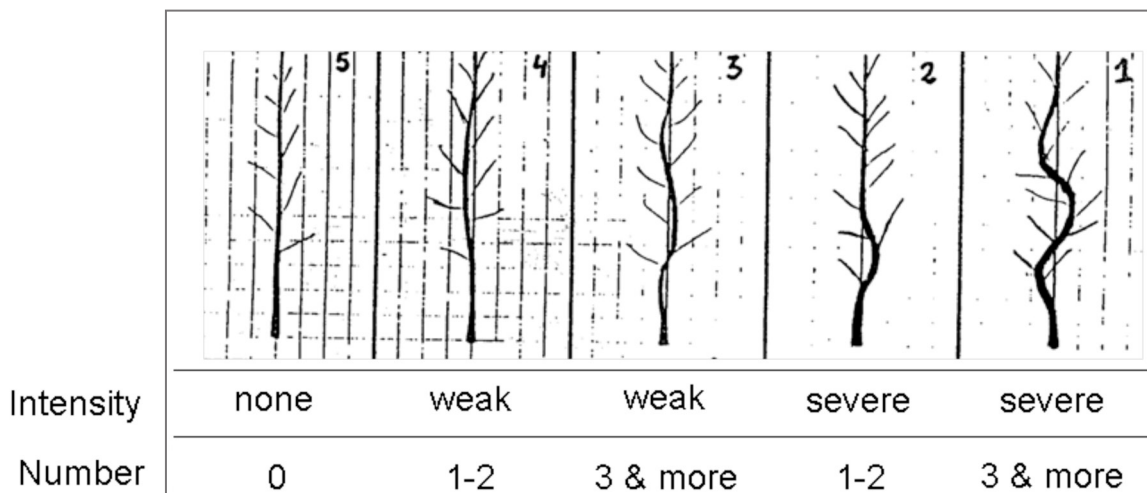


Figure 2. Scoring system for stem straightness in Conifers.

- 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;
- 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 both two different ways either in the absolute manner and in the relative one.

WAY OF ASSESSMENT

It is recommended:

- 1) - to note separately the different defects such as basal sweep, lining, bending and stem sinuosity,
 - to limit the assessment of crookedness to the part of the stem above 1.30 m (to avoid basal sweep),
 - to disregard the last 2 annual increments as, for some species, this part of the stem is heavily influenced by local weather conditions (wind) and corrections of the stem appear next season(s).

- 2) - to observe trees in two perpendicular planes, so to have a better vision of defects,
 - to have the assessment done by 2 independent observers, depending on the available resources.

Table 1. Reference genotypes for conifers chosen for their higher h^2G or H^2C values.

		Straight	Crooked
European larch	Provenance	Wienerwald (A) provenance	Gora Chelmowa(PL)
Hybrid larch	Clone	FP201DK (FR)	-

The scoring system proposed could be used for a wide range of age of trees: larch, pines, spruces and Douglas-fir (5-25 yrs, optimum when trees are around 10-15 m).

REFERENCE LITERATURE

Campbell R. K., 1965. Phenotypic variation and repeatability of stem sinuosity in Douglas-fir. Northwest science, 39 (2): 47-59.

Contrain Y., 1993. Description fine de la forme de mélèzes hybrides (*Larix x eurolepis*) en liaison avec des notations subjectives. ENSA- Rennes, 24 September 1993: 55p.

Powell G. R., 1987. Syllepsis in *Larix laricina*: analysis of tree leaders with and without sylleptic long shoots. Can. J. For. Res., 17 (6): 490-498.

Shelbourne C. J. A., Namkoong G., 1966. Photogrammetric technique for measuring bole straightness. Proc. 8th SO. Conf. For. Tree Improv. Savannah, GA: 131-136.

RESEARCH QUESTIONS

Link among score levels and economical impact on tree value;
 development of an automatic objective stem form assessment.

Morphological trait: **Basal sweep**

Conifers/broadleaves

Date: March 2011

PROTOCOL FOR ASSESSMENT OF 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, wind damage or snow creeping on steep slope). Some species are highly susceptible to this defect such as maritime pine and larch.

ASSESSMENT

A subjective scoring (presence/absence of the defect) or various objective ways of measurement of the importance of the defect are commonly practiced.

WAY OF ASSESSMENT

Basal sweep is expressed by the maximum horizontal distance between the deformed trunk and a vertical line (Figure 1). The vertical line is materialised by a pole put at the base of the stump and maintained vertical (plumb line or spirit level). The distance d is expressed in centimeters.

The deformation is observed in the plane where the defect is the most severe and up to 1.30 m.

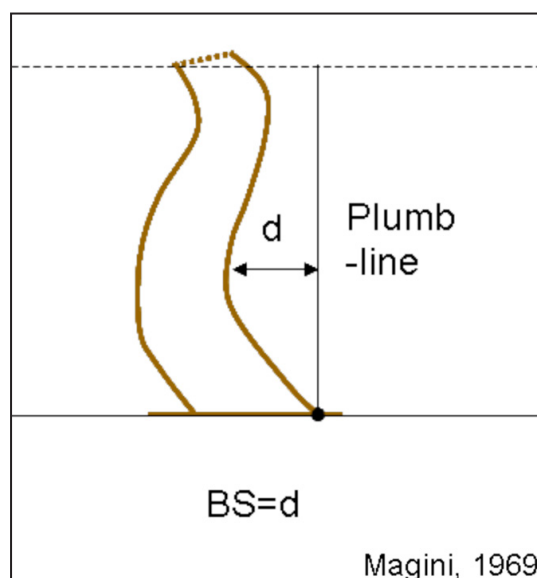


Figure 1. Scoring methods adopted for basal sweep (Magini 1969).

REFERENCE LITERATURE

Magini E., 1969. The heritability of the stem form in *Pinus pinaster* Ait. 2^{ème} Consultation Mondiale sur l'Amélioration des arbres forestiers. Washington FO-FTB 3/8: 351-360.

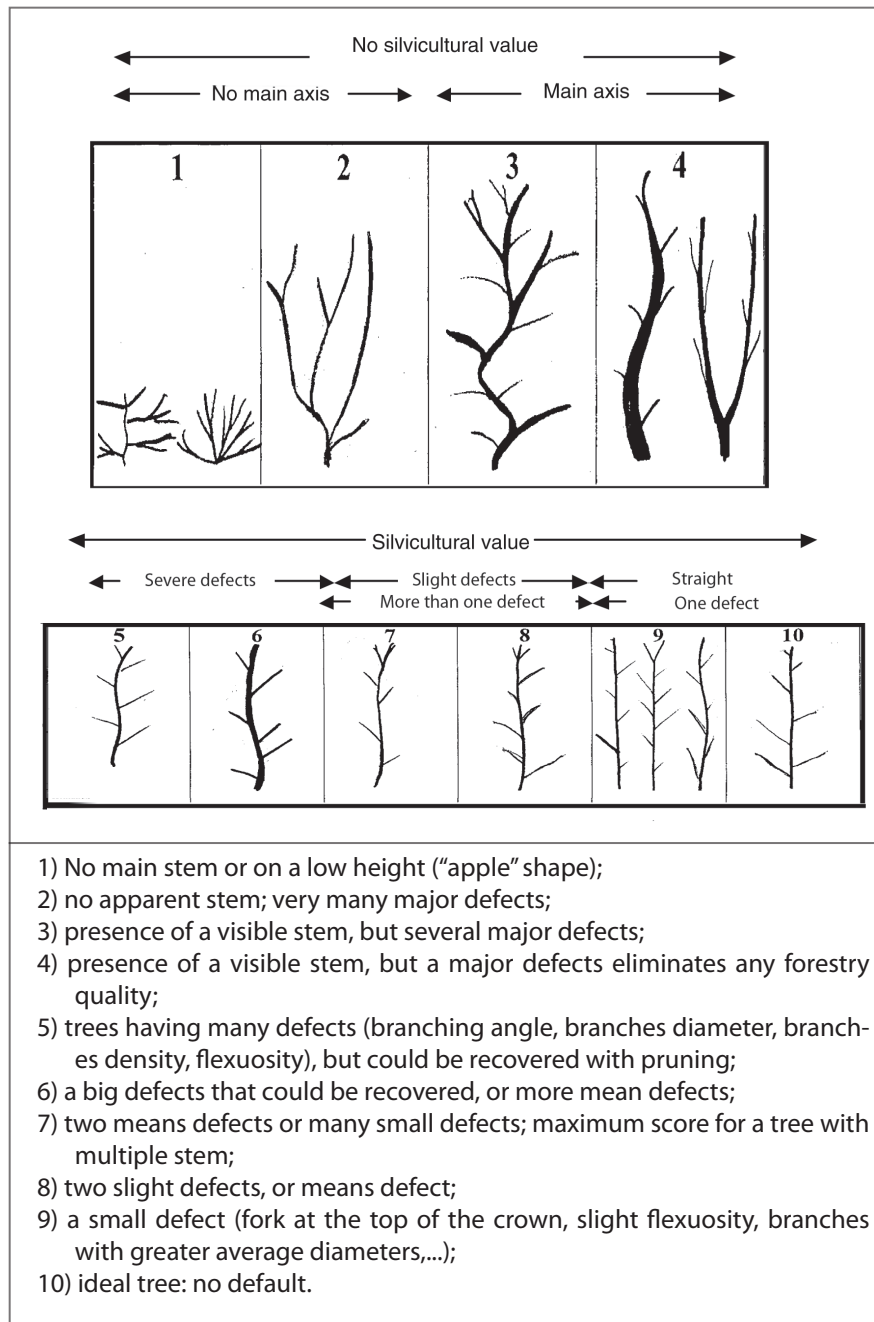


Figure 1. The scoring system used for beech and oak.

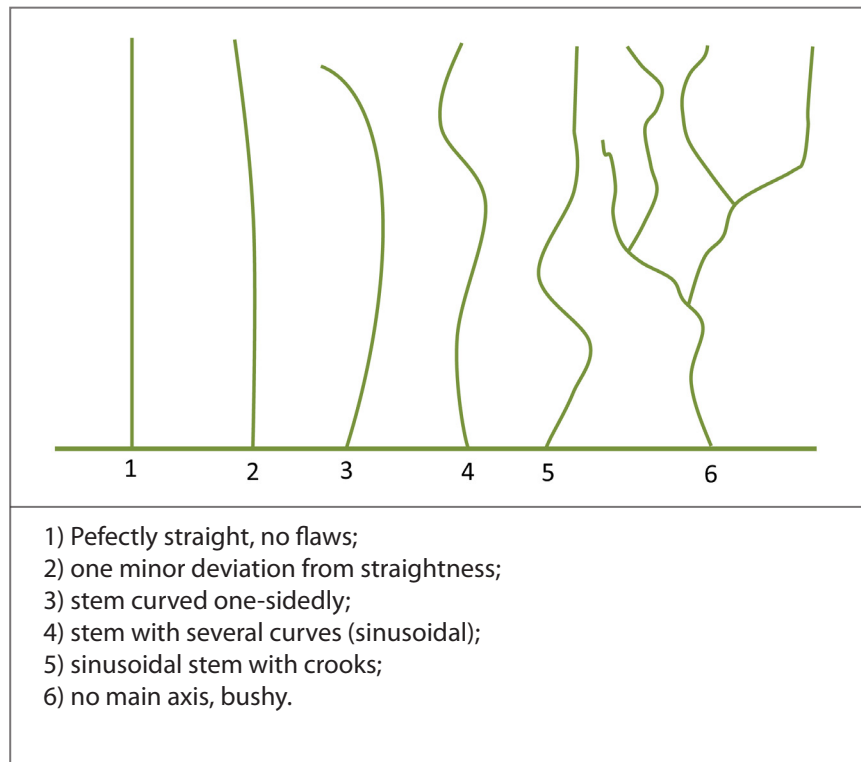


Figure 2. The scoring system used for wild cherry, ash and other scattered broadleaves.

REFERENCE LITERATURE

Ducci F., Proietti R., Calvo E., Correale F., 2005. La valutazione di cloni e provenienze. [Clone and provenance evaluation]. In: Monografia sul ciliegio selvatico (*Prunus avium* L.). [Monograph on wild cherry]. (F. Ducci ed.,) CRA ISSAR, Istituto Sperimentale per la Selvicoltura, Arezzo: 45 – 52.

Ducousso A., Guyon J.P., Kremer A., 1996. Latitudinal and altitudinal variation of bud burst in western populations of sessile oak (*Quercus petraea* (Matt.) Liebl.). *Ann. Sc. For.*, 53: 775-782.

RESEARCH QUESTIONS

Developing MAS (Marker Assisted Selection) would it be possible?

Morphological trait: **Branch angle**

Conifers/broadleaves

Date: March 2011

PROTOCOL FOR ASSESSMENT OF BRANCH ANGLE

DEFINITION

Branch angle is defined as the angle between a given branch or the mean angle of branches and the main stem axis. In case of curvature of the branches, the angle is measured at the insertion point (line y in Figure 1)

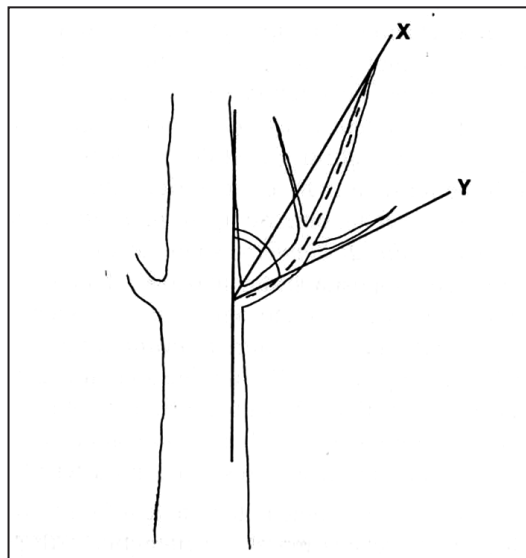


Figure 1. Insertion angle of branches compared to the stem.

ASSESSMENT

Whereas subjective scoring systems of the type (steep, medium, flat angle) are available, the definition of easily recognizable angle thresholds helps to make the observation more objective.

SCORING SYSTEM

A 5 class scoring system is proposed (Figure 2 and 3):

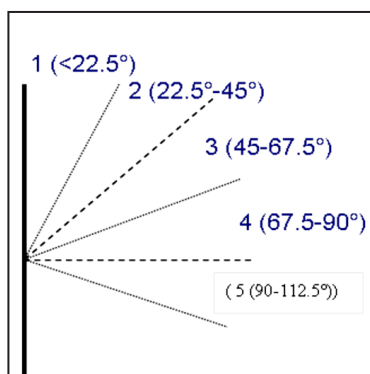


Figure 2. Scoring system proposed for evaluating the branch angle classes.

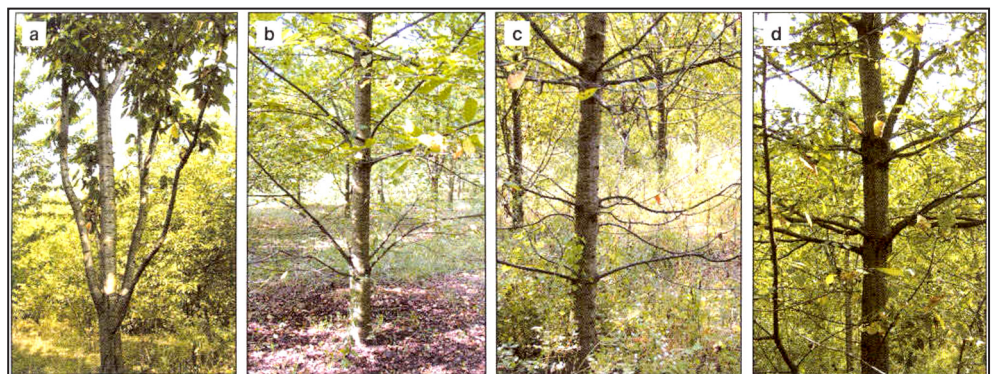


Figure 3. Examples of angle and thickness (also ref. to sheet n. 5) of branches in 4 Italian wild cherry clones: a – small angle and high thickness; b - medium angle and low thickness; c - horizontal angle and low thickness; d horizontal angle and medium thickness.

WAY OF ASSESSMENT

Actual and precise measuring of branch angles is very time-consuming, becomes unfeasible once trees have reached a certain height. Branch angle can be assessed using different tools.

Angle classes can be measured by manual goniometer or more precise measurements can be carried out with digital device to precisely measure (e.g. Nedo Winkeltronic LaserWinkeltronic®) and transfer angles – rugged, reliable and with an accuracy of $\pm 0.1^\circ$.

The scoring system can probably be used at any age but preferably beyond the juvenile stage of trees when the tree architecture stabilizes. The estimation of the mean branch angle class is commonly agreed for two whorls, i.e. located just under and just above half of the total tree height and calculation of the average of both estimates.

GENERAL RECOMMENDATIONS

The scale should be used as such with the given angle classes (and not adapted to each site).

SPECIFIC RECOMMENDATIONS

The way branches are sampled (given branch, given whorl, whole tree) should be specified in each case and may vary according to tree age and species.

Reference genotypes (population, family, clone) were chosen for their higher h^2G or H^2C values (Table1).

Table 1. Reference genotypes for branch angle.

		With typically <i>flat angle</i> branches (90°)	With typically <i>sharp angle</i> branches (less than 25°)
Wild cherry	Progenies	GEN/PAV-030; GEN/PAV-123(BE)	PB/PAV-01; PB/PAV-09(BE)
	Clones	VV04, ML11, PVN06, AP08 (IT)	n. a.

REFERENCE LITERATURE

Ducci F., Germani A., Janin G., Proietti R., Signorini G., 2006. Clone selection for wild cherry (*Prunus avium* L.) with special reference to some traits used. Bozzano M., Rusanen M., Rotach P., Koskela J., 2006. (compilers). Euforgen – Noble Hardwoods Network, report of VI (9-11 June 2002, Alter do Chao, Portugal) and VII meeting (22 – 24 April 2004, Arezzo, Italy), IPGRI, Rome, Italy: 53 – 60.

RESEARCH QUESTIONS

Link between score levels and economical impact on tree value.

Morphological trait: **Branch thickness**

Conifers/broadleaves

Date: March 2011

PROTOCOL FOR ASSESSMENT OF BRANCH THICKNESS

DEFINITION

Branch thickness refers to the quality of branching in terms of branch diameter. Branches with thicker diameters will leave knots with a larger area which will decrease wood quality.

ASSESSMENT

Branch thickness is evaluated either by direct measurement of (a sample of) branches or with a subjective scoring system (thin, medium, thick). A significant improvement is provided when branch thickness is related to stem diameter or when you use ratios as mean diameter of branches in the whorl/Stem diameter.

SCORING SYSTEM

The scoring system proposed is a 4-score scale with 1 (worst) and 4 (best). Branch thickness is related to stem diameter at the level of the branch.

The figure 1 shows an example for assessment of branch thickness in conifer species. An example of branch thickness types in broadleaves is visualised in previous procol nr. 4, figure 3.





			
1	2	3	4
$\frac{1}{2}$ of stem diameter	$\frac{1}{2}$ to $\frac{1}{3}$	$\frac{1}{3}$ to $\frac{1}{4}$	Less than $\frac{1}{4}$

Figure 1. Scores for branch thickness in Conifers (pictures from IBL - PL).

WAY OF ASSESSMENT

Specific recommendations

Common whorls or pseudo-whorls (1 or 2) are first agreed upon and branch thickness evaluation will consider only these branches. The mean score for all branches of a given (pseudo-) whorl is given relative to the stem diameter just above the whorl.

There are no *a priori* age limits for the assessment as far as whorls can be clearly seen, but probably the best period for conifers is between 5 and 20 yrs and for broadleaves between 3 and 10 years. The estimation of the branch thickness class for two whorls has to be located just under and just above half of the total tree height and the average of both estimations must be calculated.

Specific recommendations

For wild cherry (example on protocol nr. 4): the only real way to control branch thickness is planting wild cherry in mixed plantations with shelter species or in forest.

Reference genotypes (population, family, clone) were chosen for their higher h^2G or H^2C values (Table 1).

Table 1. Reference genotypes for branch thickness.

		With typically <i>thin</i> branching	With typically <i>coarse</i> branching
Wild cherry	Progenies	GEN/PAV-026 ; GEN/PAV-037 (BE)	PB/PAV-01; GEN/PAV-062 (BE)
	Clones	ML11 (IT)	n. a.

REFERENCE LITERATURE

Ducci F., 2005. Metodi di individuazione e selezione di materiali di base. [Methods for the survey and selection of basic materials]. In: Monografia sul ciliegio selvatico (*Prunus avium* L.). [Monograph on wild cherry]. (F. Ducci ed.,) CRA ISSAR, Istituto Sperimentale per la Selvicoltura, Arezzo, Italy: 33 – 36.

RESEARCH QUESTIONS

Any known industrial thresholds in knot size integrated in selection index?

Morphological trait: **Apical dominance**

Conifers/broadleaves

Date: October 2010

PROTOCOL FOR ASSESSMENT OF APICAL DOMINANCE

DEFINITION

Apical dominance refers to the normal pattern of annual height growth when the main leader is formed year after year in the main axis of the stem. In several species prone to several growth cycles [*St-John's sprout*, *polycyclism*, *lamma's shoot*], defects can be observed due to loss of dominance. Sub-terminal twigs can then elongate to such an extent that forks can be formed. It appears in both broadleaves and conifers.

ASSESSMENT

Several subjective scoring systems have been proposed. They usually distinguish between cases where apical dominance is respected (a main axis still exists even if lateral branches take over in some ways) or not, and cases where multiple leaders definitely take over.

SCORING SYSTEM

Broadleaves (e.g. wild cherry; Figure 1)

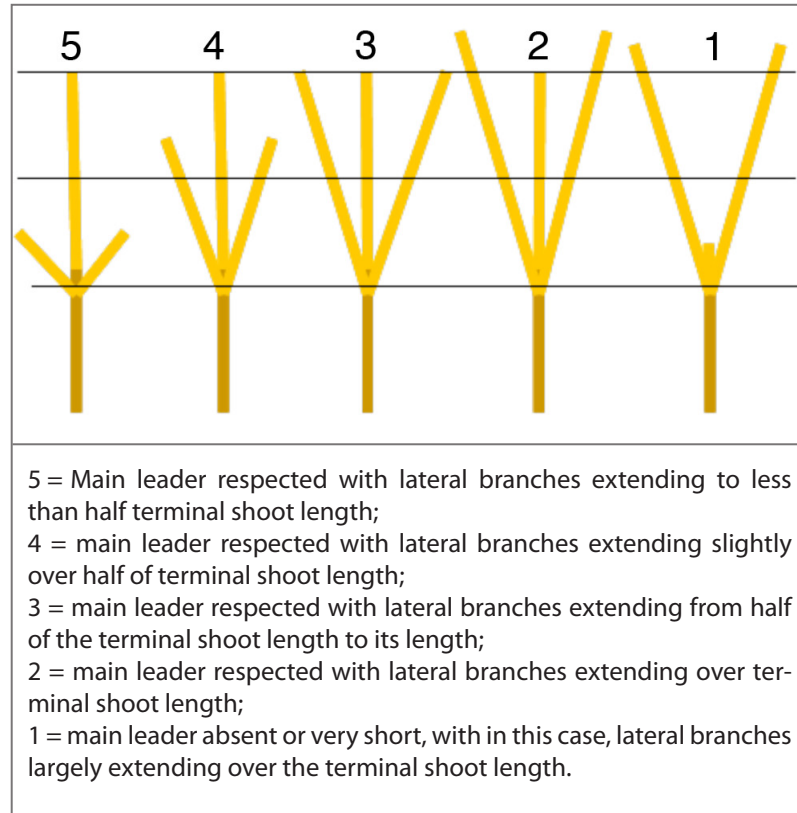


Figure 1. Scoring system for apical dominance in broadleaves trees.

Conifers (e.g. Douglas fir, Firs, Larch; Figure 2):

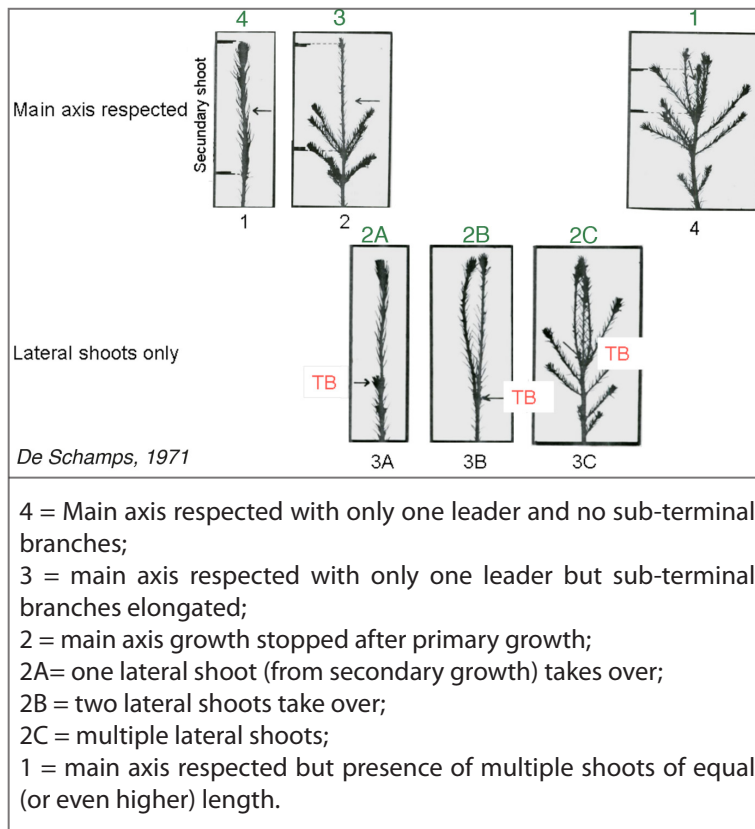


Figure 2. Scoring system for apical dominance in conifers (TB: Top Bud). Scores within the picture box are those used by De Schamps (1971). Scores outside the box are defined in legenda.

WAY OF ASSESSMENT

The top of each tree will be carefully observed and noted according to the scoring scales above. Care should be particularly taken for conifers to properly observe primary and secondary shoots. Between growth cycles, growth can momentary stop with formation of a bud before second growth starts, or it can just slow down for a more or less short period before second growing. In this case, needles or scars of needles will appear locally more dense.

Assessment of polycyclism, being a juvenile characteristic, should concentrate on young trees (on average up to 10 years).

Reference genotypes (population, family, clone) were chosen for their higher h^2G or H^2C values (Table 1).

Table 1. Reference genotypes for apical dominance.

		With <i>strong</i> apical dominance	With <i>weak</i> apical dominance
Wild cherry	Progenies	GEN/PAV-042; PB/PAV-13 (BE)	GEN/PAV-003; GEN/PAV-025 (BE)

REFERENCE LITERATURE

De Schamps, J. 1971. Pousses d'aout du Douglas. Rapport annuel. Association Foret-Cellulose (AFOCEL), Paris: 173-217.

Ducci F., 2005. Metodi di individuazione e selezione di materiali di base. [Methods for the survey and selection of basic materials]. In: Monografia sul ciliegio selvatico (*Prunus avium* L.). [Monograph on wild cherry]. (F. Ducci ed.,) CRA ISSAR, Istituto Sperimentale per la Selvicoltura, Arezzo: 33 – 36.

Ducci F, Veracini A., 1990. Criteri di scelta e sistema di valutazione di fenotipi superiori nel miglioramento genetico di latifoglie a legname pregiato. [Evaluation and selection criteria in the frame work of the phenotypic selection and improvement and breeding of noble hardwoods]. Ann. Ist. Sper. Selv., Arezzo, Italy. Vol. XXI: 57 – 80.

Morphological trait: **Forking**

Conifers/broadleaves

Date: October 2010

PROTOCOL FOR ASSESSMENT OF FORKING

DEFINITION

A *fork* is a frequent defect in several species, genetically inherited and/or accidental (damage to terminal bud or last annual increment). Compared to a *ramicorn* (spike knot), 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, and making a steep angle with the trunk.

ASSESSMENT

Presence vs. absence of fork is the most straightforward method. Counting of forks is another possibility, much more informative than the previous one as repetitive forking might suggest a genetic control of the defect. The major inconvenient of both methods is that according to the height at which the fork appears, the defect can be judged detrimental or not. Many authors have thus included in their scoring the location of forking (depth as a proportion of height, height).

SCORING SYSTEM

A combination of the methods above is proposed in the following 7-scores scale (Figure 1). It combines the presence/absence of forks, their number and their position relative to total tree height.

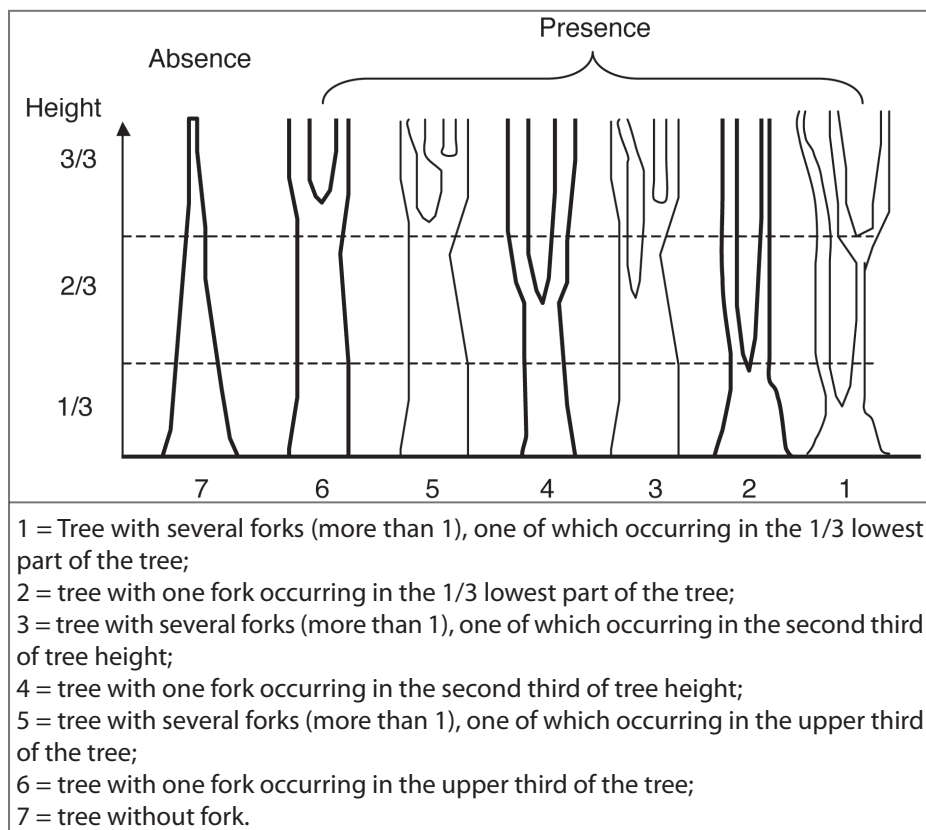


Figure 1. Scoring system for forking defects.

WAY OF ASSESSMENT

Each tree is observed carefully and given a note according to the scale described above. Probably the scale is best adapted for mature trees but could be used as early as 10-15 yrs old.

Reference genotypes (population, family, clone) were chosen for their higher h^2G or H^2C values (Table 1).

Table 1. Reference genotypes for forking.

	With typically <i>non forked</i> trees	With typically <i>forked</i> trees
Wild cherry Progenies	GEN/PAV-062 ; GEN/PAV-082 (BE)	GEN/PAV-080 ; PB/PAV-12 (BE) n.a., in Italy, forked trees are discharged at phenotypic selection step

RESEARCH QUESTIONS

N. a.



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TREEBREEDEX RESEARCH INFRASTRUCTURE NETWORK 2006 -2011

(European project CT2006-026076)

ADAPTIVE TRAITS



Adaptive traits: **Phenology**

Definitions - recommendations

Date: October 2010

PROTOCOLS FOR ASSESSMENT OF PHENOLOGY TRAITS

GENERAL DEFINITIONS

Adaptation: it is the adjustment of a gene pool of a population to a given environment (i.e. ecotypes of spruce adapted to low altitude climate or to high altitude mountain climate) (Nanson, 2004).

Tolerance: it is the ability of a genotype to preserve its fitness under the pressure of a damage factor. It is genetically settled and fixed by the evolutionary forces, allows each species to occupy a given ecological niche in a given habitat.

Phenotypic plasticity: it is the asymmetric response of genotypes to extreme events. It can be defined as the property of a given genotype to produce different phenotypes in response to distinct environmental conditions. It can be considered at several levels: Temporal plasticity, Spatial plasticity, Within-tree plasticity, Trans-generational plasticity, etc.

Phenology: it is an important aspect of *adaption*. It is the study of the timing of periodic phenomena such as flowering, growth initiation, growth cessation, water flows etc., cambial and physiological activity, especially as related to seasonal changes in temperature, photoperiod, etc. (Wright, 1976; Nanson, 2004).

Phenology traits (bud break, bud burst or bud flush) are conditioned by several biological and environmental factors (chilling requirements, forcing temperatures, etc.) necessary for launching the processes but they are also under strong genetic control.

Local tree conditions might also interfere on the speed of developmental phases, such as tree height and health stage, water availability in soil, etc..

They are important selection criteria as they might condition stem form in case of stress, frost (mostly late frost) damage, but also they affect growth potential in relation to the growth season length.

SPECIFIC DEFINITIONS FOR PHENOLOGY

Bud break, bud burst or bud flush: corresponds to the period spanning from dormant bud up to shoot elongation. It is conditioned by several environmental factors (chilling requirements and forcing temperatures) necessary for launching the process but it is also under strong genetic control.

Bud set: corresponds to the period when elongation stops and new buds (preformed in most Conifers) are ready to cope with winter climate.

Flowering phenology: is the period when male and female flowers develop.

Hardening: is the period needed by the elongated shoots to conclude the lignifications processes before the end of vegetation period.

Late frost resistance: related to observation of bud break.

Leaf senescence: corresponds to the period when progressive changing of leaf/needle color is observed.

Requirement of chilling temperatures: for deep dormancy release, it is usually measured by chilling units (CU), the number of hours passed under limit of temperature (usually 5°C).

Shoot elongation: after the bud scales are open, the shoot starts the elongation phase. It is concluded when the length growth stops.

Shoot frost hardness: is the period needed by the elongated shoots to conclude the lignifications processes before the end of vegetation period.

Other traits rarely used:

Cambial activity – to study the start date and the annual growth phase by using anatomy of the cambial activity in the ring growth (picking micro-cores method, Italy).

Cell cycle analysis – to study early growth initiation and late growth cessation in apical buds (Sweden).

Spike knots - as indication of autumn or winter frost damage (Lithuania).

ASSESSMENT

A subjective scoring system is commonly used, but also quantitative methods can be used: developmental stages from dormant bud to elongation are described. Observation usually concerns the terminal buds or in the case of tall trees the terminal bud of lateral branches is noted. Always the same portion of the crown should be monitored on all trees of the test.

Based on funding resources, observations are either conducted:

- at a given periodicity (every 2 days) during the flushing period with each tree noted at each observation;
- or 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;
- or only once or twice during the flushing season.

WAYS OF ASSESSMENT

Two cases are presented:

Case 1: For genotype ranking (method by default)

By default, the observation of bud burst will be done only once during the flushing season, when around half of the trees have reached the mid-score (3), that means that some trees are still at an earlier stage and some already at a later stage.

A preliminary notation on a sample of trees across the trial (e.g. on one or two diagonals) or on reference genotypes can be used to better fit the assessment period.

Case 2: For comparison of flushing dynamics among genotypes

The observation of bud burst will be done at regular intervals (*twice a week, more often* according to the needs) along the flushing season, starting soon enough to capture the early stage and ending late enough to catch the last stage.

In this case, at a given date, all trees will be observed and recorded. The observation will stop when all trees have reached stage 5.

A person is on average able to sample 500-1250 trees per day according to the size of trees and consequent difficult for scoring.

Monitoring can be repeated only once or for several years according to the aims of the experiments.

Concerning this kind of traits, their potential can be fully realized only in a series of provenance plots along an altitudinal/latitudinal (climatic) gradient(s).

Phenology characters are practically relevant indicators of the adaptability and adaptedness of forest trees (Table 1). They are directly related to the growth and to the tree architecture. There is a correlation between them and the

Table1. Criteria concerning the phenology monitoring.

	Ranking genotypes	Relation with environmental parameters (ex. heat-sum)	Dynamics of flushing	Constraints
Periodically, each stage, all trees at each assessment	Y	Y	Y	Every 2-3 days, all trees are recorded: time-consuming, long period of observation.
Periodically, one stage, only trees recently flushed at each assessment	Y	Y	N	Every 2-3 days, only trees having reached a given stage are recorded and materialised in the field (painting) to avoid its assessment next time: easier, less-time consuming; long period of observation.
Once, all trees at a time	Y	N	N	Trees are assessed only once: rapid and simple

frost damage, which incidence is expected to increase with the climate change. A series of assessments on the same individuals over more years allows concluding about the climate development.

For leaf senescence, a subjective scoring system allows classifying trees according to a grid representing different stages of tree yellowing.

GENERAL RECOMMENDATIONS

In all cases, the terminal bud alone will be noted but care should be paid that it is alive and healthy. In case of defects, a sub-terminal bud can be assessed instead.

Observation is best done on small trees like in nursery (but attention to transplantation shock) or in field experiments when trees are lower than 3-4 meters and optimally 2-3 meters. Assessment of taller trees requires the help of binocular which makes the observation more delicate and in any case more time-consuming.

The site conditions can change requirements of traits to be monitored in different countries so a standardized method should be adopted.

Each tree is given a score (from 0 (1) to 5). Observation is preferably done block by block in one single day. A given observer must achieve complete blocks (observer effect confounded with block effect in statistical analysis).

For shoot elongation and shoot frost hardening: the trees must not be too high so you can reach the upper part of the crowns without major problem.

For freeze testing the test temperature is critical to assess genetic variation (too low or too high test temp. means poor resolution between genetic entries).

PRESENTATION OF RESULTS

Data will be provided in a two-way table with genotypes and dates of observations: cells will include the scores.

REFERENCE GENOTYPES

As suggested by C. Bastien (INRA, Orléans-France), 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 most test sites as possible.

Adaptive traits: **Phenology**

Bud break in *Abies* species

Date: October 2010

PROTOCOL FOR ASSESSMENT OF BUD BREAK IN *ABIES* SPECIES

SCORING SYSTEM

The scoring methodology below has been developed and tested for *Abies* spp..

Bud break (CRA - SEL, IT)

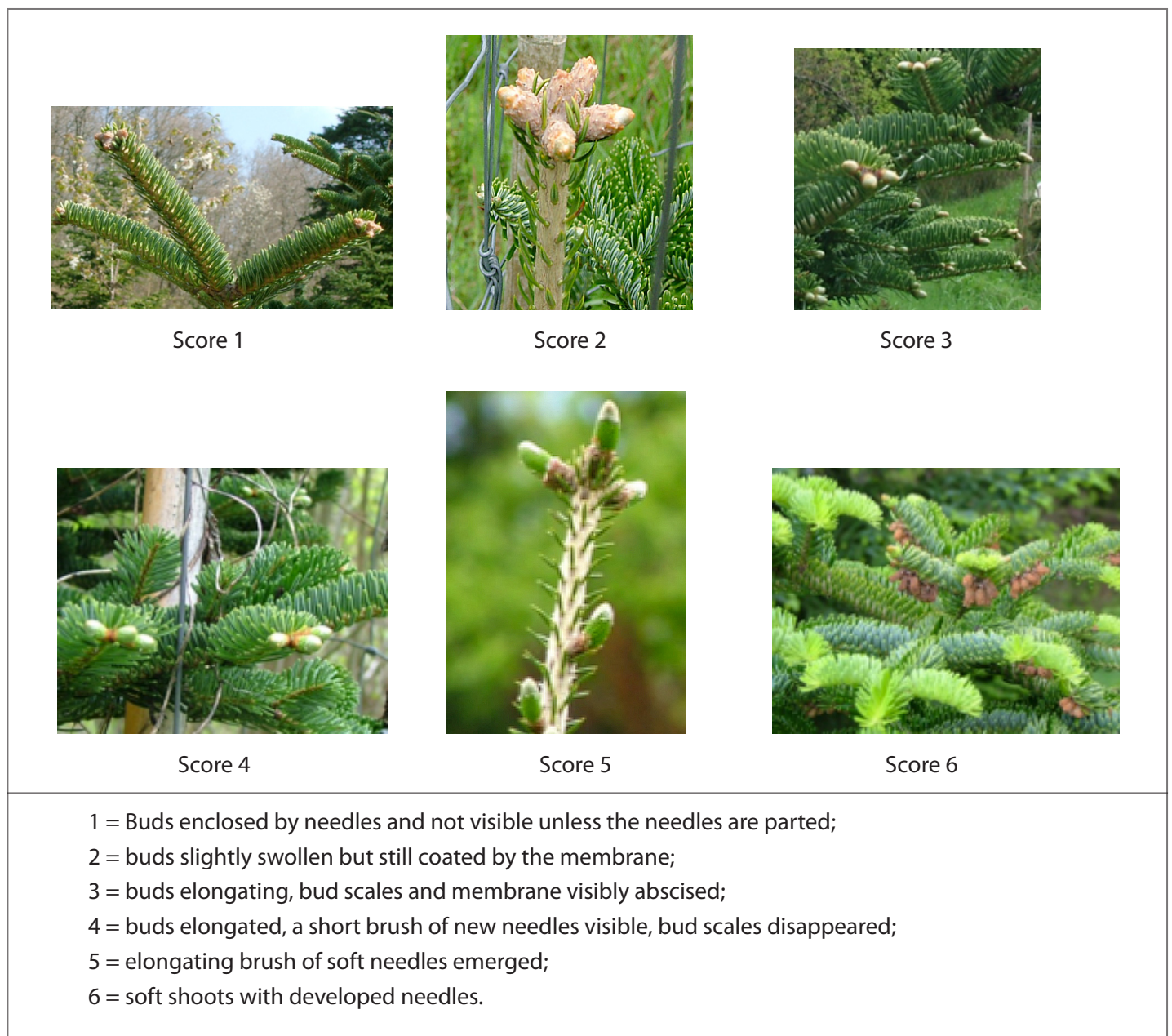


Figure 1. Scoring system developed by CRA - SEL (IT) for *Abies nebrodensis*.

The observation of *bud break* will be done at regular intervals (*twice a week, more often?*) along the flushing season, starting soon enough to capture the early stage and ending late enough to catch the last stage. It should be correlated with chilling units accumulated along the winter and according to the length of day when international trials are considered. The observation will stop when all trees have reached the final stage 6.

Adaptive traits: **Phenology**

Bud burst in larch

Date: October 2010

PROTOCOL FOR ASSESSMENT OF BUD BURST IN LARCH**SCORING SYSTEM**

Scoring system have been provided for the survey on adaptive traits (Figure 1).

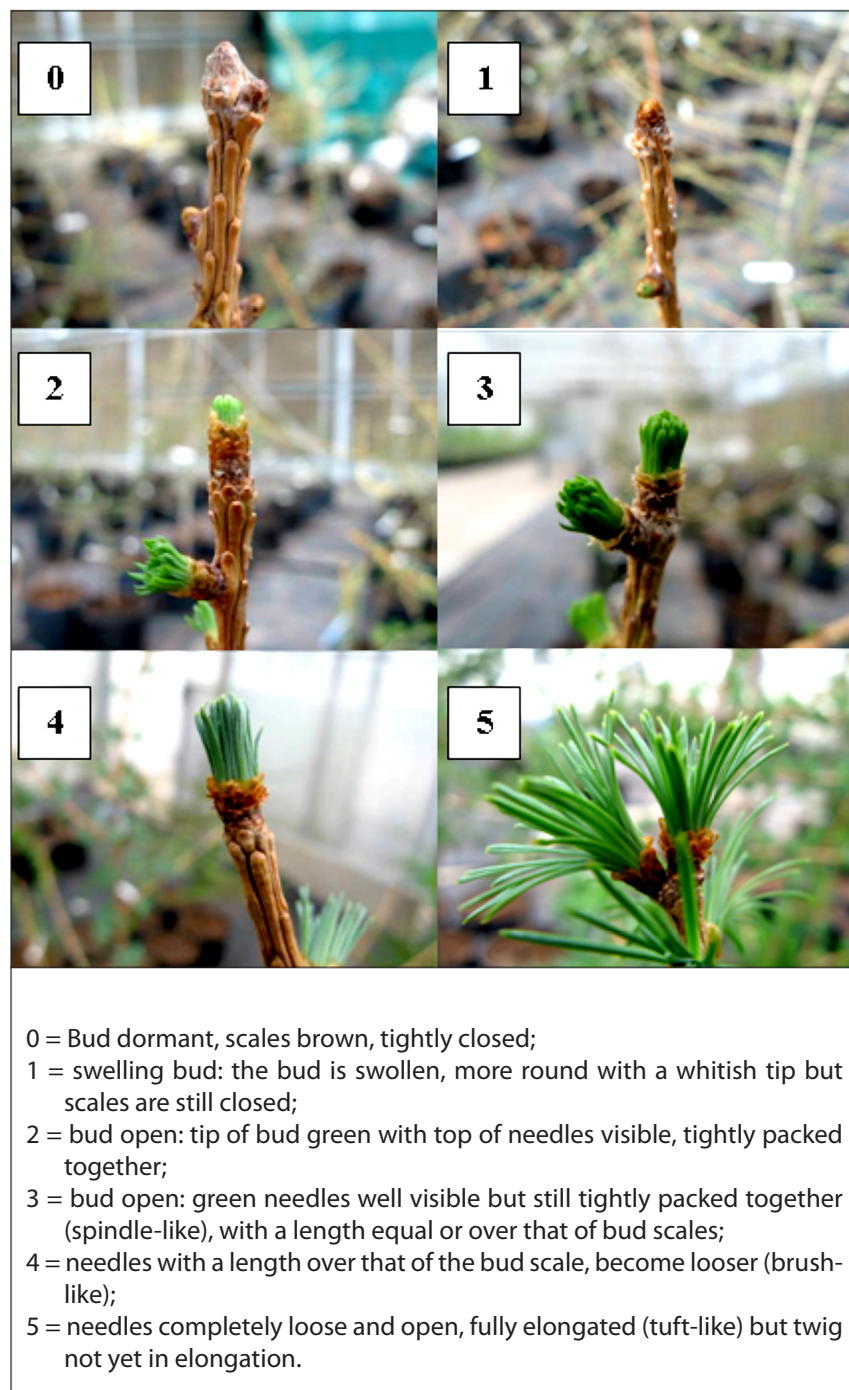


Figure 1. Flushing scores in larch (INRA - Orléans; photos L.E. Pâques, 2009).

Adaptive traits: **Phenology**

Bud burst, bud break (*budset, shoot elongation*), termination of growth in Spruce

Date: October 2010

PROTOCOL FOR ASSESSMENT OF BUD BURST, BUD BREAK AND TERMINATION OF GROWTH IN SPRUCE

SCORING SYSTEM

Bud burst

The developmental stage of the lateral bud is scored using the scale of Krutzsch (1973; Figure 1).

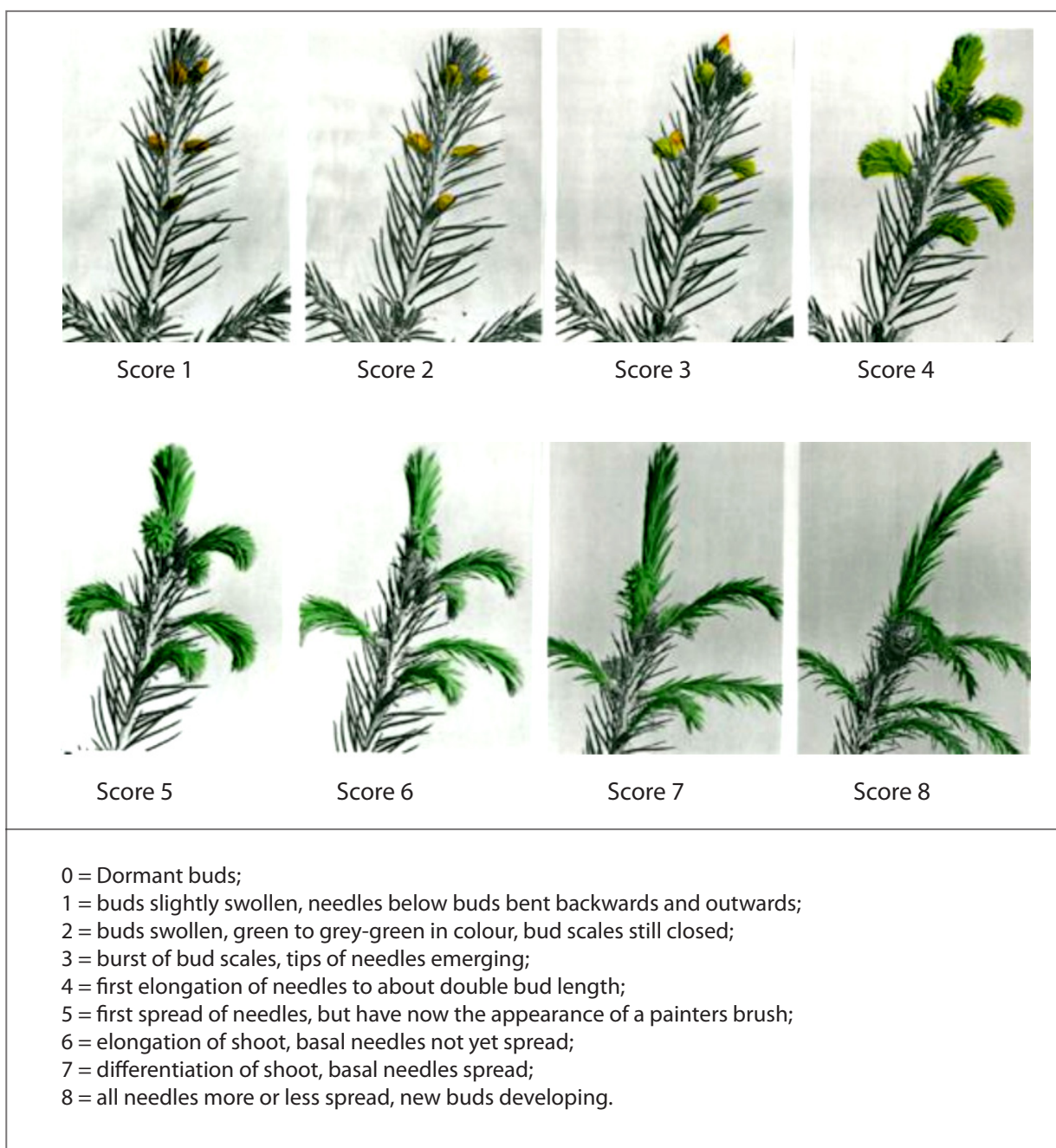


Figure 1. The scoring method adopted for Spruce.

Bud break-*Picea* spp. (Figure 2)

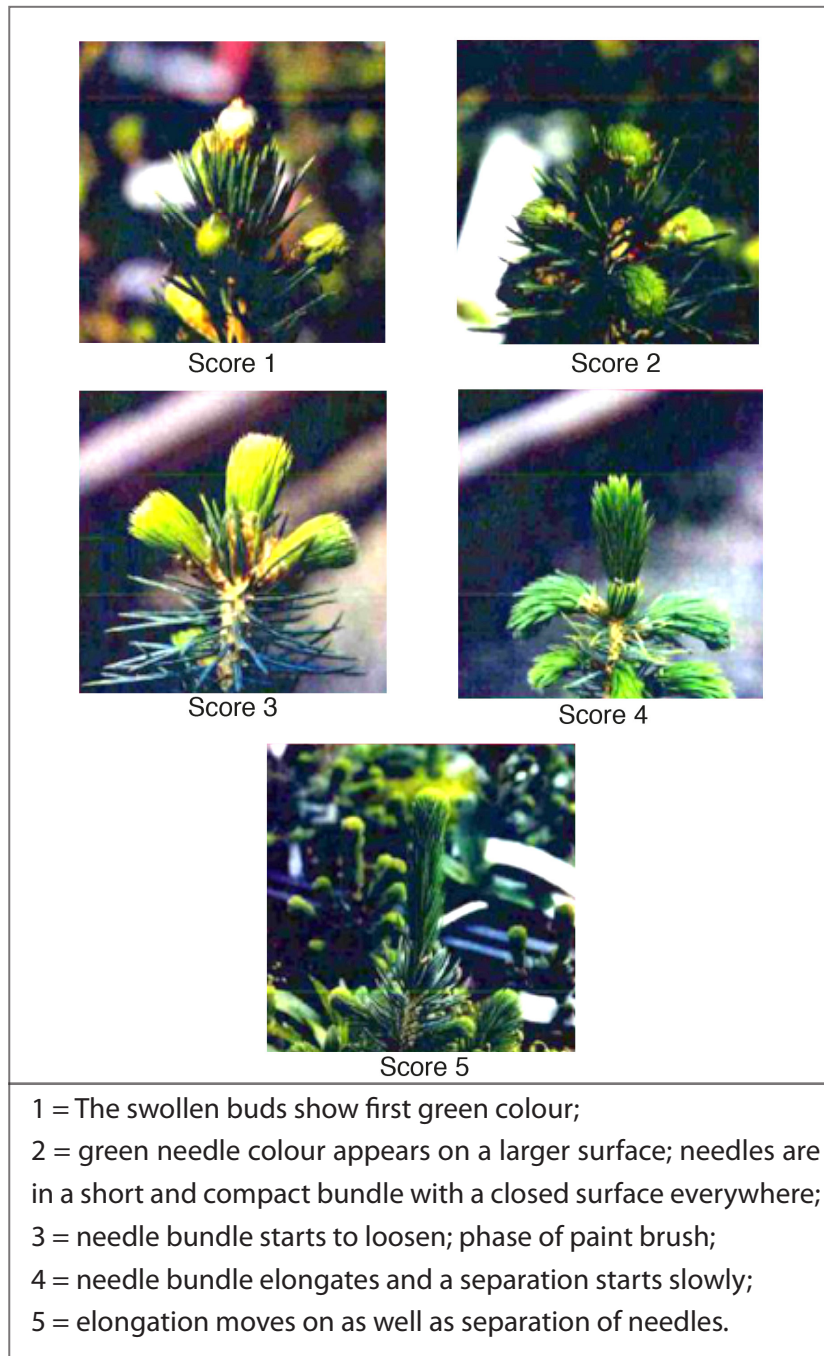


Figure 2. Assessment of Bud set in *Picea* species.

WAY OF ASSESSMENT

Traits can be monitored at different ages. In general young trees are preferred, when the top bud can be seen on the leader shoot then the budburst of the top bud can be assessed on the leader shoot (nursery to 7-8 years old in the test) according to 8 score scale similar to Krutzsch (1973).

Scales used at different ages:

Seedlings

- 1 = Buds enclosed by needles, and not visible unless the needles are parted;
- 2 = buds beginning to swell;
- 3 = buds beginning to elongate, the scales slightly separated, but not retracted;
- 4 = buds swollen and more elongated, bud scales retracting;

- 5 = a short brush of new needles visible, bud scales abscising, a "hat" of bud scales remains on the top of the new shoot;
 6 = a longer brush of needles emerged, all bud scales have disappeared.

Adult trees

- 0 = Buds enclosed by needles, and not visible unless the needles are parted;
 1 = buds swelling but still coated by the membrane;
 2 = buds elongating, bud scales and membrane visibly abscised, a "hat" of bud scales on the top of the new shoot;
 3 = buds elongated, a short brush of new needles visible, bud scales disappeared;
 4 = brush of needles emerged;
 5 = soft shoots developed, ca 5 cm long;
 6 = shoot straight, needles dark green.

Termination of growth (Figure 3)

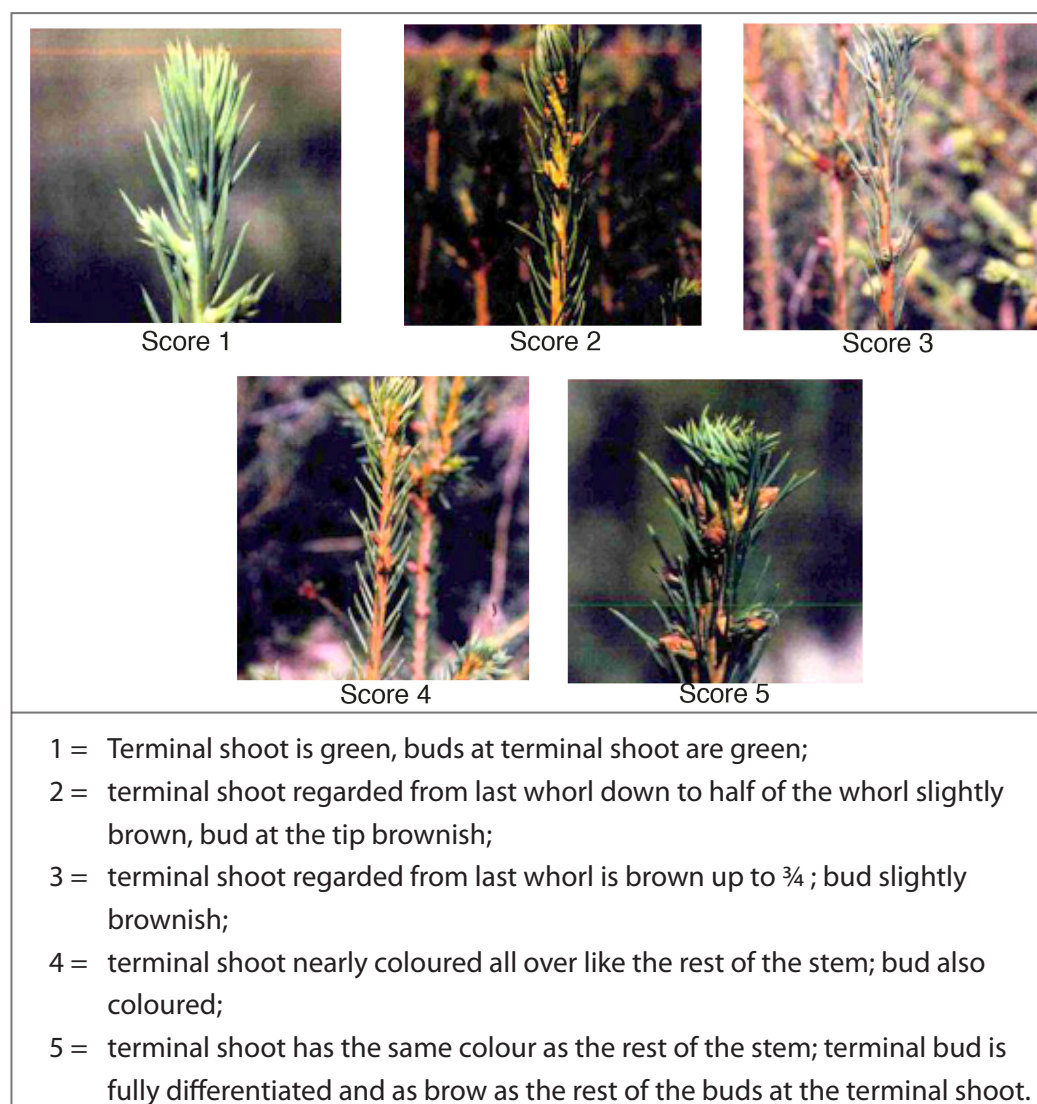


Figure 3. Assessment of termination of growth in *Picea* species.

Adaptive traits: **Phenology**

Bud break, shoot elongation

Pinus sylvestris

Date: October 2010

PROTOCOL FOR ASSESSMENT OF BUD BREAK AND SHOOT ELONGATION IN *PINUS SYLVESTRIS*

SCORING SYSTEM

Bud break and shoot elongation phenology

Scoring the phenological stage according to the 0 to 5 scale defined by Debazac (1966; Figure 1).

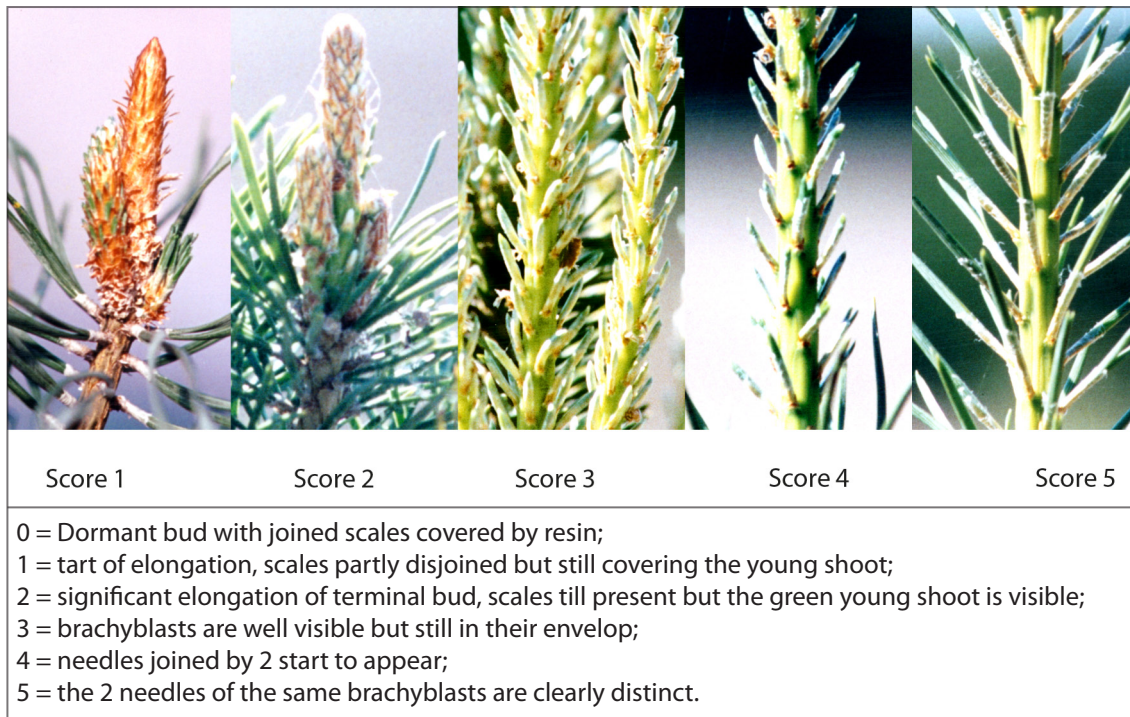


Figure 1. Scoring system adopted for bud breaking and elongation in *Pinus sylvestris*.

Adaptive traits: **Phenology**

Bud break, bud set

Pseudotsuga menziesii

Date: October 2010

PROTOCOL FOR ASSESSMENT OF BUD BREAK AND BUD SET IN *PSEUDOTSUGA MENZIESII*

SCORING SYSTEM

Bud break (Figure 1)

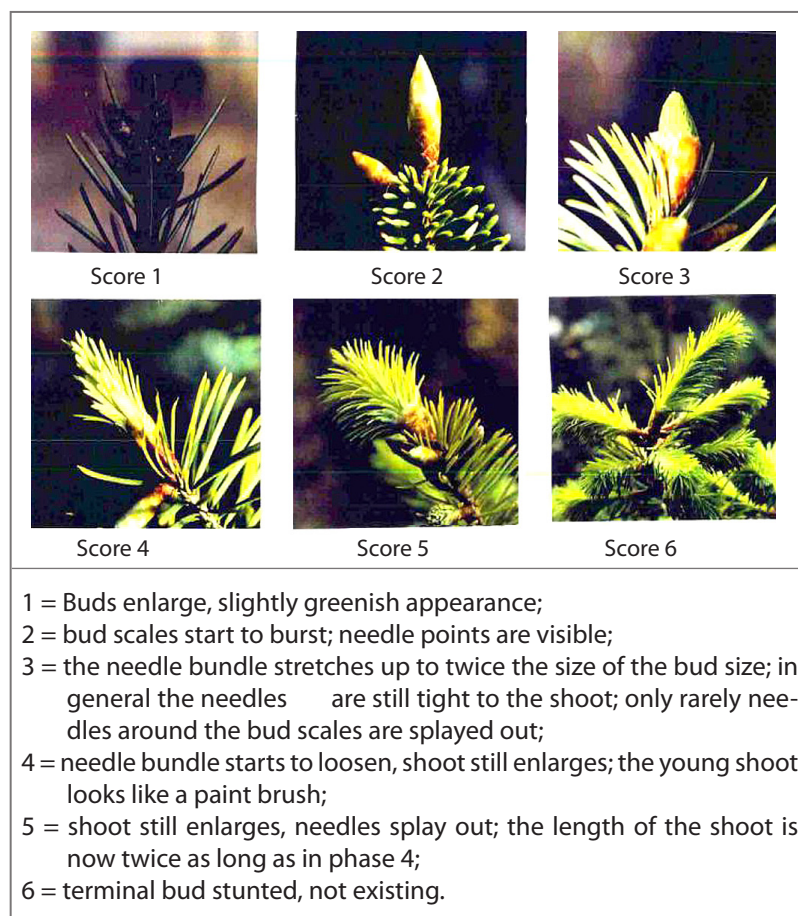


Figure 1. Scoring system adopted for bud break on Douglas Fir.

Termination of growth (plants in nursery; Figure 2)

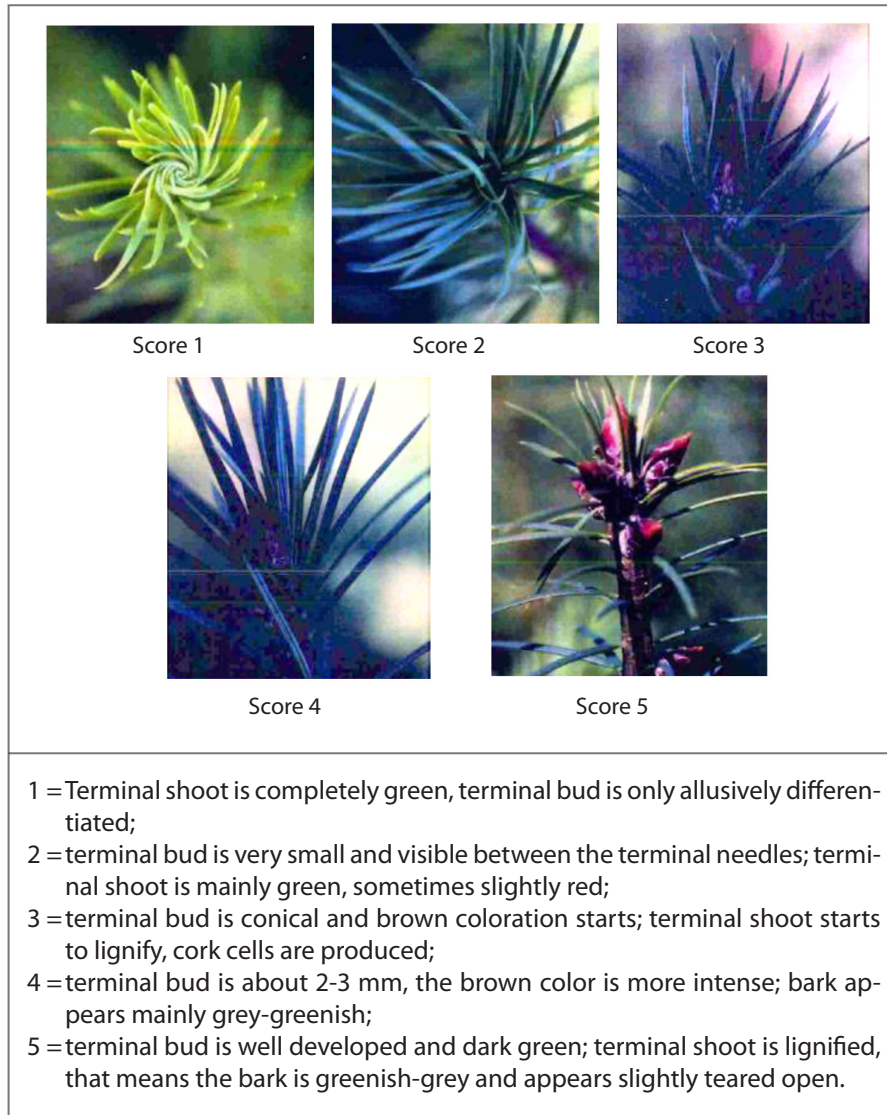


Figure 2. Scoring system adopted for bud setting/growth termination on Douglas Fir

Adaptive traits: **Phenology**

Bud break, bud set, termination of growth

Populus spp.

Date: October 2010

PROTOCOL FOR ASSESSMENT OF BUD BREAK, BUD SET AND TERMINATION OF GROWTH IN *POPULUS* SPECIES

SCORING SYSTEM: DESCRIPTION

In Poplars methods and ways for assessing phenology traits are widely standardized, due to international agreements.

Bud break - Method 1

Scoring the phenological stage according to the 0 to 5 scale defined for *P.nigra* in the EUFORGEN technical guidelines (Figure 1). This scale has been tested on the three species and their hybrids.

Between 1 to 3 assessments the same year of the same experiment in order to catch early, medium and late flushing genotypes. About 1000 trees per person per day can be assessed for Bud break.

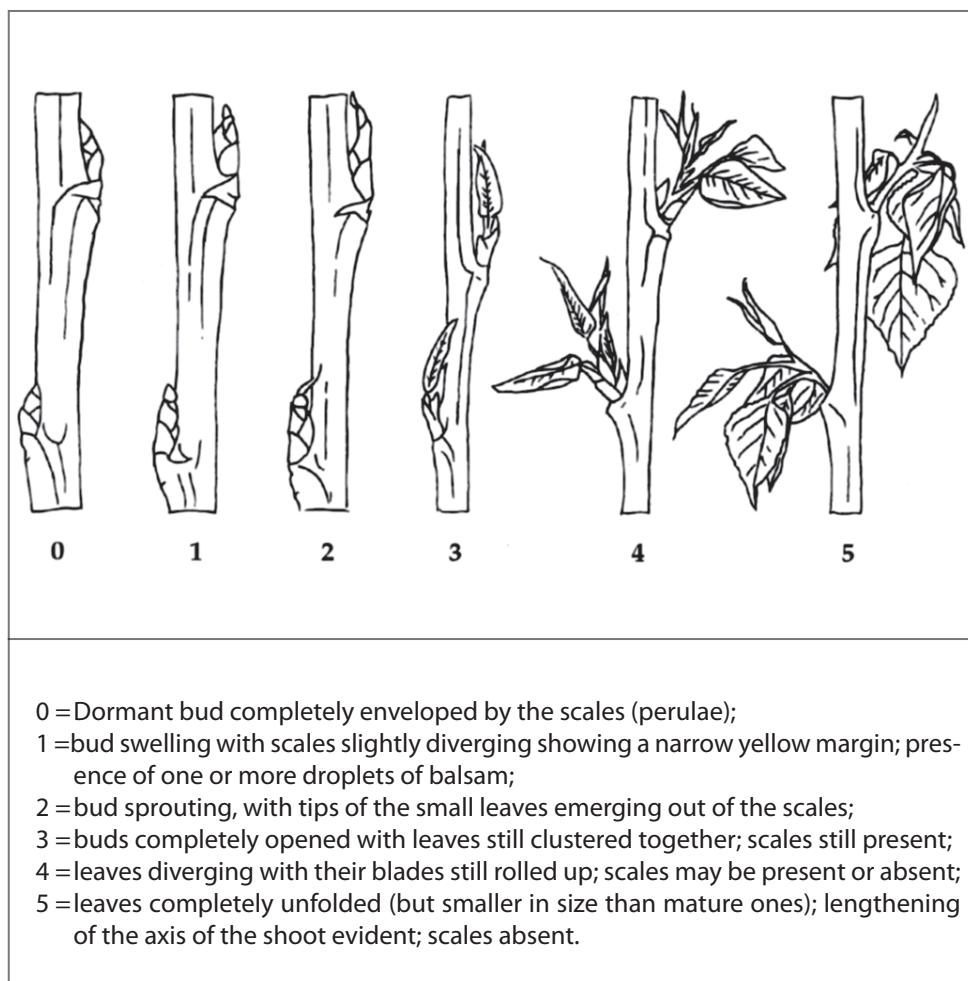


Figure 1. The Euforgen scoring system adopted for bud burst of poplars (Turok *et al.*, 1996).

Bud break - Method 2

Bud development stages

0 = Dormant buds;

1 = bud swelling;

2 = bud breaking, scales are opening, the tips of the uppermost new leaves are visible;

3 = bud breakdown, scales still present, young leaves emerging from the upper part of the bud, but still joined;

4 = leaf opening stage, young leaves involuted;

5 = leaves wide opened, shoot elongation.

Budset

Between 6 to 12 assessments the same year of the same experiment in order to catch early, medium and late flushing genotypes. Every two-days or twice a week. About 1000 trees per person per day can be assessed for Bud Set.

Budset - Method 1

Scoring the phenological stage according to the 0-3 scale (Figure 2) defined first for *P. nigra* by Rohde and Ruttink (2004) and adapted on other species and hybrids by C. Bastien at INRA (2005). Between 1 to 5 assessments the same year of the same experiment in order to catch early, medium and late flushing genotypes. It is more difficult to assess than bud break and needs some technical training.

0-3: Budset scale initially defined on *P. nigra* by Rohde and Ruttink (2004).

0-3: Bud set scale adapted to inter-specific Poplar hybrids (Figure 3).

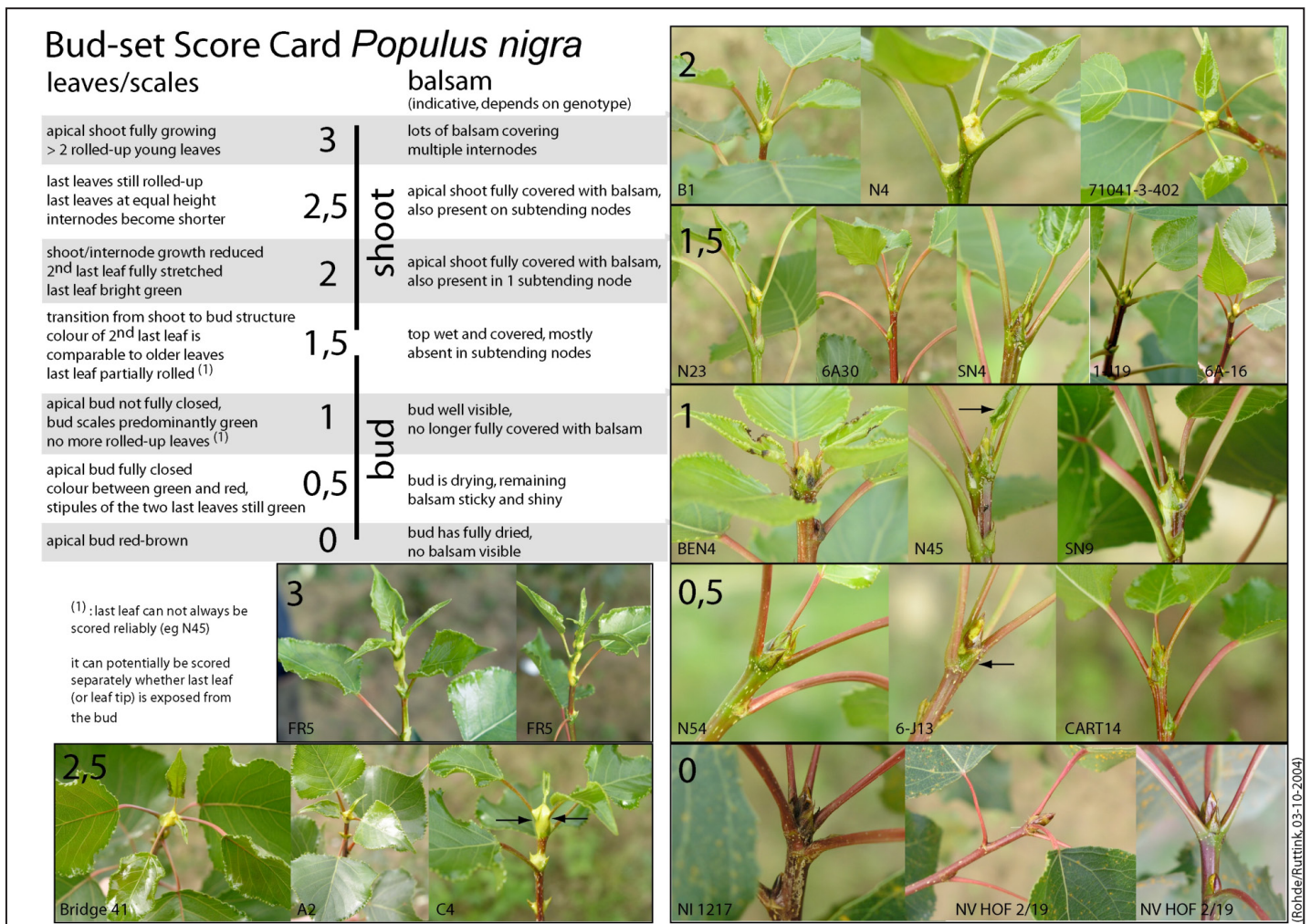


Figure 2. Bud set scoring system adopted for lack poplar (Project FP6-POPYOMICS).

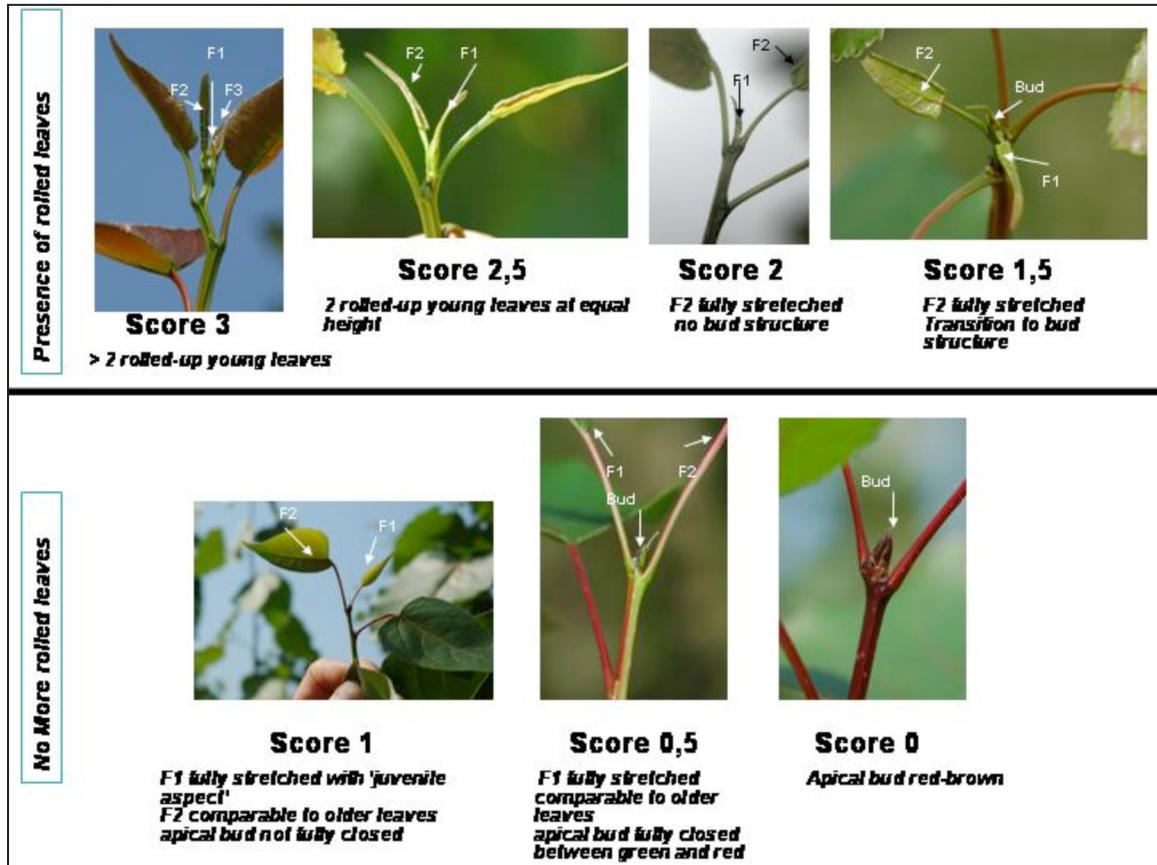


Figure 3. Bud set scoring system adopted on hybrid poplars.

Budset - Method 2

- 0 = if terminal bud has not reached stage 1.5;
- 1 = if terminal bud has reached stage 1.5.

Termination of growth

A 6 score scale of is used in Germany for Termination of growth in Poplars. This method is to be preferred because simpler and rapid (Figure 4).

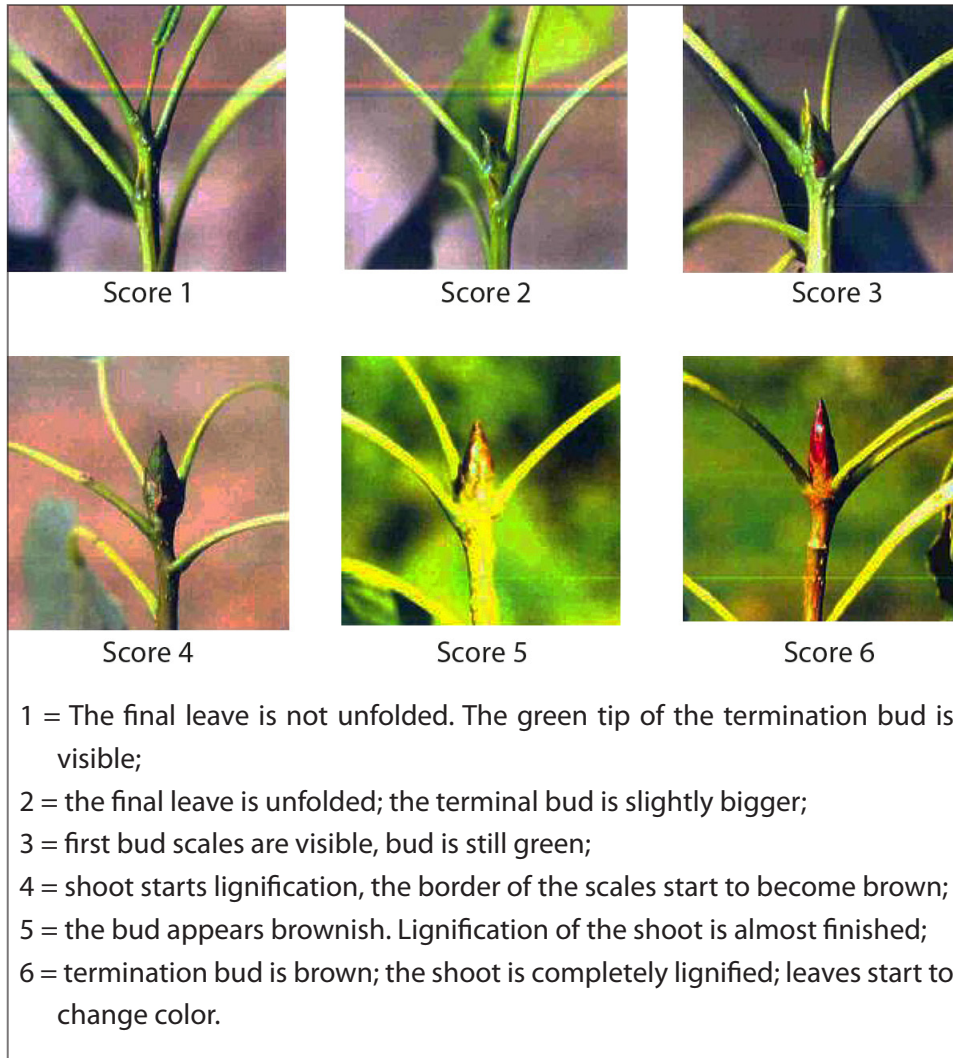


Figure 4. Bud set scoring method adopted in Germany for hybrid poplars.

Adaptive traits: **Phenology**

Budbreak

Prunus avium

Date: October 2010

PROTOCOL FOR ASSESSMENT OF BUD BREAK IN *PRUNUS AVIUM***SCORING SYSTEM: DESCRIPTION**

In wild cherry methods and ways for assessing phenology traits are only partially standardized, due to international projects as Always (AIR3). Photographs and drawings allow a relatively fast assessment (Figure 1).

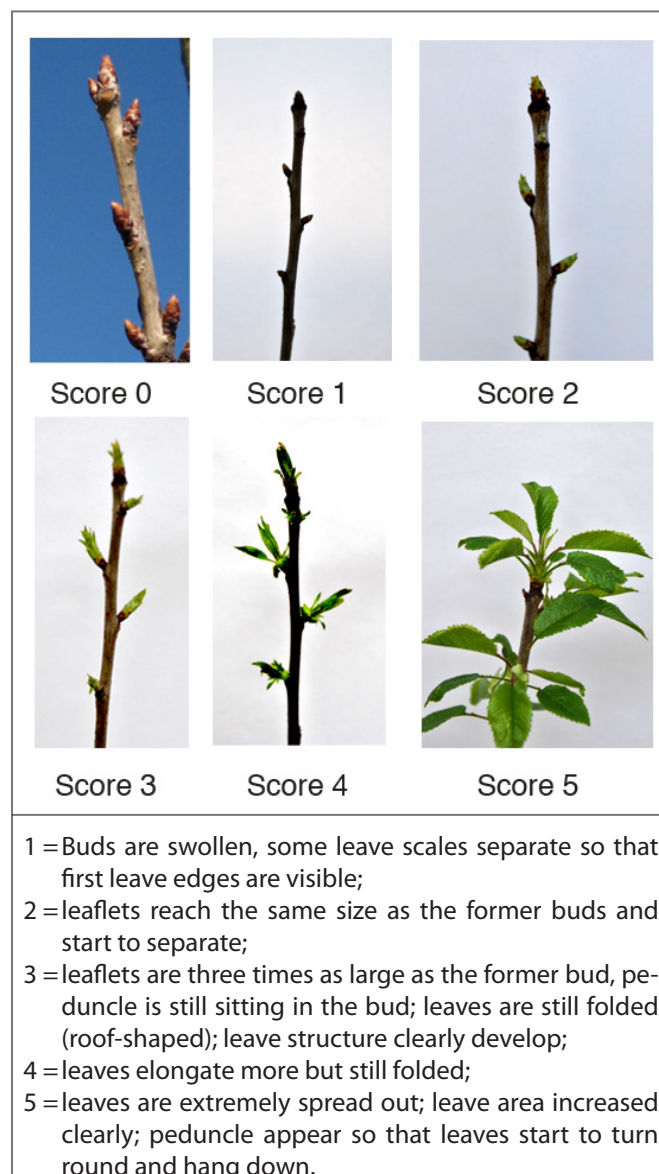


Figure 1. Scoring method adopted for wild cherry on bud break.

WAY OF ASSESSMENT

In northern countries this trait is important within the scope of climate change and important adaptive trait re-

lated to late frost damages. Anyway this trait is also meaningful related to survey genetic variation in a species showing very low intra specific levels of differentiation.

Even if the evaluation can be highly subjective, photographs allow a relatively fast assessment. A team of 4 people can measure about 600 trees per day.

Bud break scoring system in Belgium, France, Italy and other countries.

The observation of bud break will be done at regular intervals varying according to the needs and the sites along the flushing season, starting soon enough to capture the early stage and ending late enough to catch the last stage. It should be correlated with chilling units accumulated along the winter and according to the length of day when international trials are considered. The observation will stop when all trees have reached the final stage 5.

In other cases, observation can be carried out at given dates (once, twice or three or more times per year), trees will be observed and recorded at the phase they are.

Adaptive traits: **Phenology**

Scoring system for leaf senescence

Date: October 2010

SCORING SYSTEM FOR LEAF SENESCENCE IN LARCH

The following grid has been proposed by Migliavacca *et al.* (2008; Figure 1).

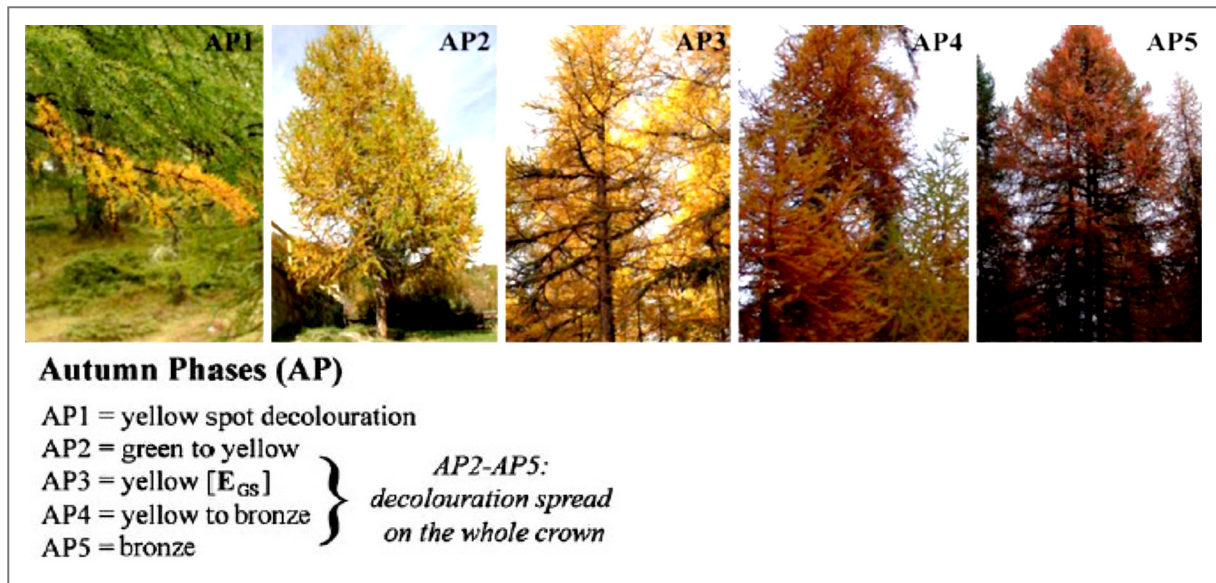


Figure 1. Scoring system in larch (Migliavacca *et al.*, 2008).

WAY OF ASSESSMENT

For *bud set*, the terminal bud alone will be observed but care should be paid that it is alive and healthy. In case of defects, a sub-terminal bud can be assessed instead.

Observation is best done on small trees like in nursery (but attention to transplantation shock) or in field experiments when trees are lower than 3-4 m. Assessment of taller trees requires the help of binocular which makes the observation more delicate and in any case more time-consuming.

For *leaf senescence (yellowing)*, the whole crown of trees is observed: leaf yellowing usually starts in larch from bottom to top of crown and from inside towards outside the crown.

Observation is probably more reliable on more mature trees and in any case on trees with a more fully developed crown than on smaller trees (confirmation needed).

PRESENTATION OF RESULTS

Case 1: data will be provided for each individual tree including the date of bud set or the date when the senescence reference stage was reached;

Case 2: data will be provided in a two-way table with genotypes and dates of observations: cells will include the senescence scores.

RESEARCH QUESTIONS

Impact of height of trees on bud set?

Relationships with environmental factors? Possibility to have indirect assessment methods;

meaning of bud set in larch compared to leaf senescence with relation to the end of cambial activity?

Adaptive traits: **Phenology**

Flowering phenology (male and female)

Abies spp.

Date: October 2010

FLOWERING PHENOLOGY IN *ABIES* SPECIES

SCORING SYSTEM: DESCRIPTION

In *Abies* species, with special reference to grafted or rooted cuttings seed orchards, where flowers monitoring is easier, both female and male flower phenology can be scored. Both scales are based on sets of 5 reference scores.

This traits should be monitored at least twice/week, by the same staff or person .

Female flower phenology (Figure 1).

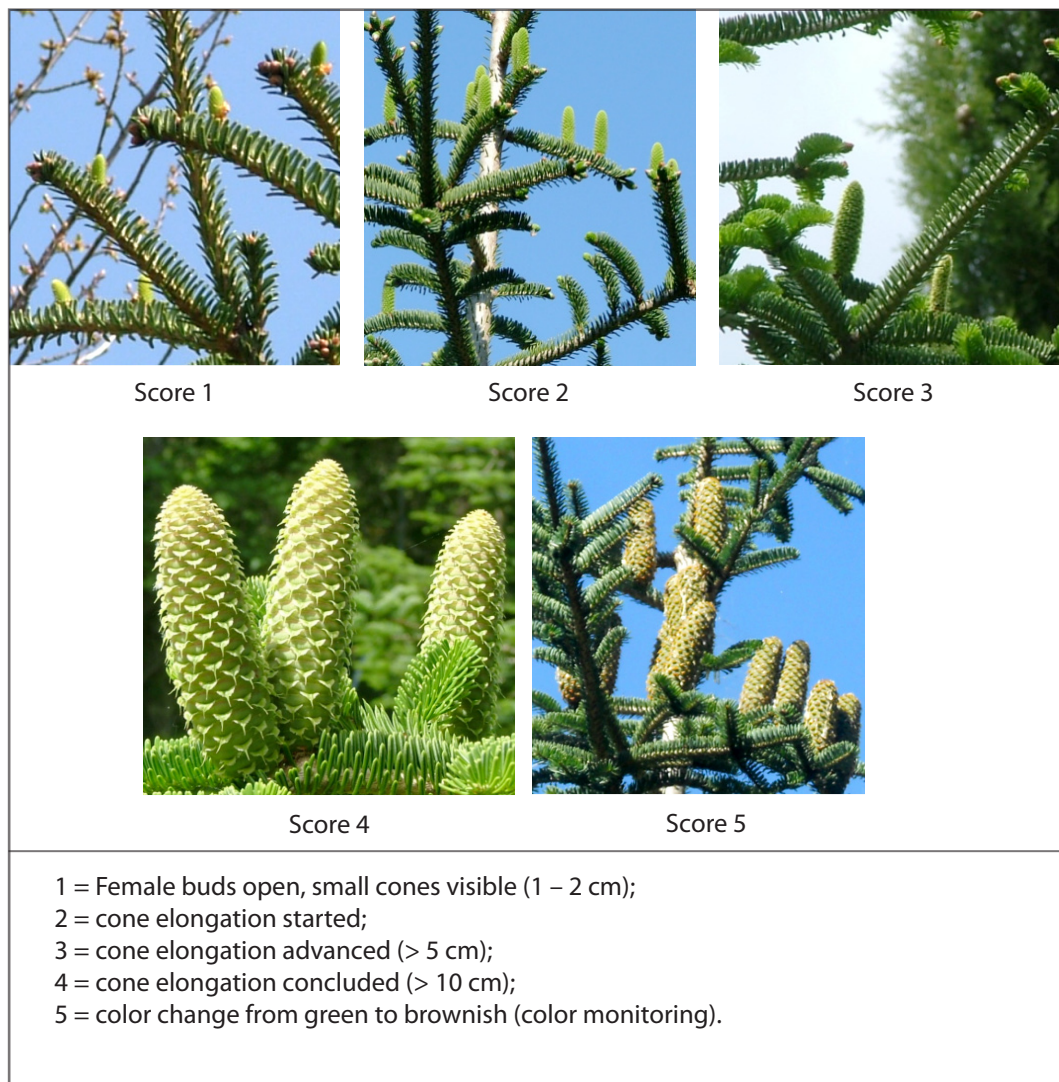


Figure 1. Scoring system to assess female flower phenology tested on *Abies nebrodensis* .

Male flower phenology

The observation can be carried out at given dates (once, twice or three or more times per year), trees will be observed and recorded at the phase they are (Figure 2).



Figure 2. Scoring system to assess male flower phenology tested on *Abies nebrodensis*.

Adaptive traits: **Phenology**

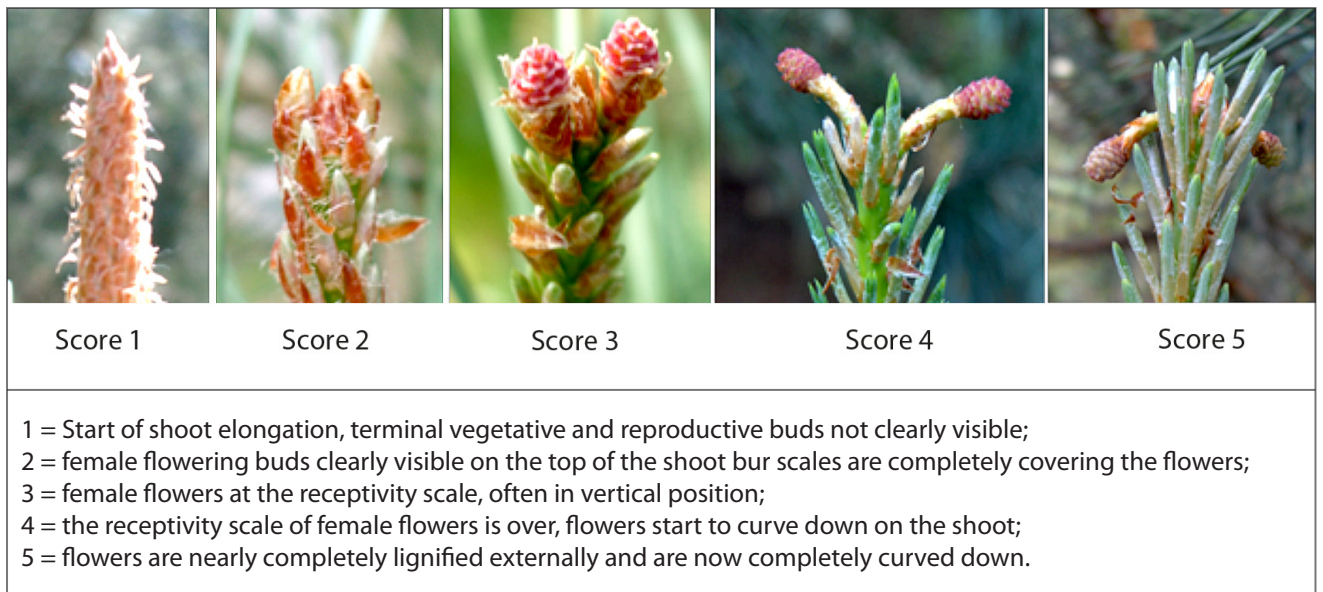
Flowering phenology (male and female)

Pinus spp.

Date: October 2010

FLOWERING PHENOLOGY TRAITS IN *PINUS SYLVESTRIS***Female flowering phenology**

Scoring the phenological stage according to a 1 to 5 scale (Figure 1).

Figure 1. Female flower phenology scoring method in *Pinus sylvestris*.

Male flowering phenology

Scoring the phenological stage according to a 1 to 5 scale (Figure 2).

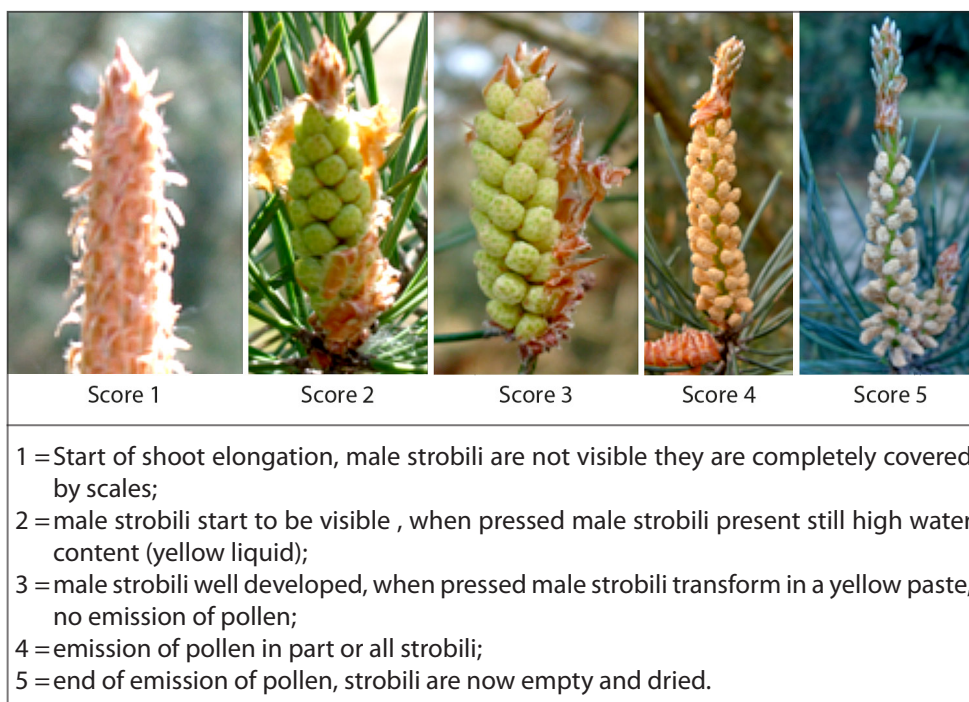


Figure 2. Scoring system adopted for male flower phenology on *Pinus sylvestris*.

Adaptive traits: **Phenology**

Flowering phenology (male and female)

Populus spp.

Date: October 2010

FLOWERING PHENOLOGY IN *POPULUS* SPECIES

SCORING SYSTEM

In Poplars, methods and ways for assessing phenology traits are widely standardized, due to international agreements.

Method

Scoring the phenological flowering stage according to the visual 1-5 scale defined by Marc Villar (INRA). Between 2 to 5 assessments the same year of the same experiment in order to catch early, medium and late flowering genotypes (Figure 1).

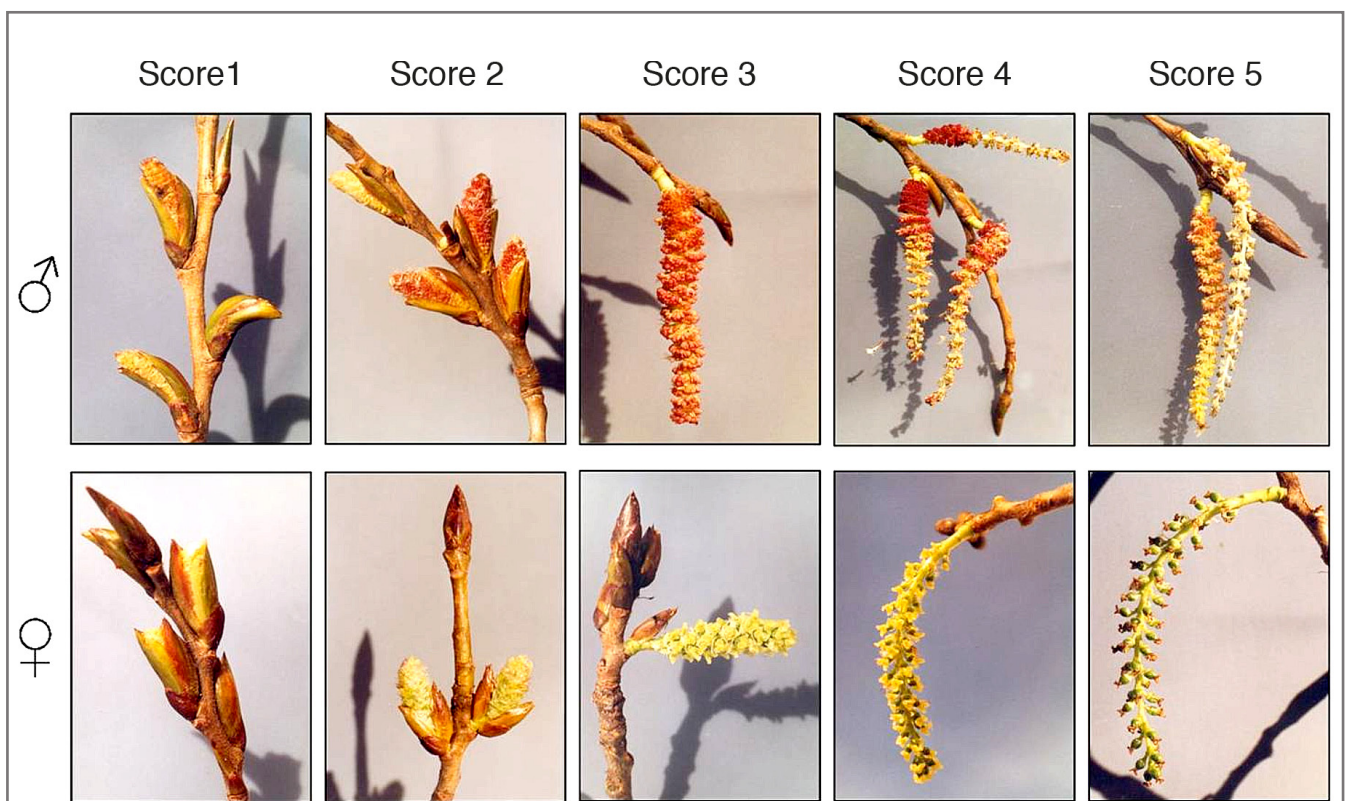


Figure 1. Female and male flowering stages scored for poplars.

Adaptive traits: **Phenology**

Flowering and fruit phenology

Prunus spp.

Date: October 2010

FLOWERING PHENOLOGY AND FRUIT RIPENING IN *PRUNUS AVIUM*

SCORING FLOWERING

The method proposed is rapid and partially standardized; it allows detection of genetic variation, characterization of clones and, in seed orchards, it allows knowing which clone can cross or can be crossed with each other. The scoring scale used is the same as the standard used for sweet cherry. Only one estimate per tree is done at age 5.

Percentage of open flowers is evaluated every 2 days. The crown is divided into 5 strata and the share (%) of fully opened flowers is recorded for each stratum.

A unique stage of flowering is observed, called "open flowers": petals can be seen open and not fallen.

1% means that around 10 flowers are open; counting continues to 5, 10, 20, 30, 40, 50, 60, 70, 80, 90%.

The starting date is estimated when breaking of flowering buds of the earliest flowering trees in the trial is recorded. The ending date when wilting of flowers of latest flowering trees in the trial. The total period of observation is on average 5 weeks.

Scoring is carried out once/year, repeated 3 years in collections.

When clones are monitored, 3-4 ramets/clone can be used.

In collections and clonal archives about 300 trees can be scored in half a day. In seed orchards, about 120 trees in 1 hour can be measured. A team of 4 people can monitor about 1200 trees.

INTEGRATED METHODS FOR PHENOLOGY ASSESSMENT

Recently, in Italy (CRA - SEL, P 12), an integrated approach for phenology traits evaluation was tested by integrating the different methods of scoring applied for bud break, flowering and ripening of fruits (Scheme 1). That was tested in order to follow all the phenological period in clone collections with the aim to characterize clone and to increase discrimination among materials within wild cherry, where neutral genetic variation is evident at individual level only. The method allowed also to pick up relationships between those traits and temperature amounts as CU (chilling units), needed by each genotype for starting the different phases.

Scores and references were also mutuated from the experienced world of fruit crop trees breeders, where phenology/adaptive maps of major genotypes were realized (Figure 1).

This system can be expansive in terms of time/men and it is suggested to be applied within collections with special attention to reference clones.

During validation tests in 2009, 2010 and 2011, it revealed differentiation between high altitude and low altitude and southern/northern materials concerning needs in flower bud dormancy induction in late summer early autumn.

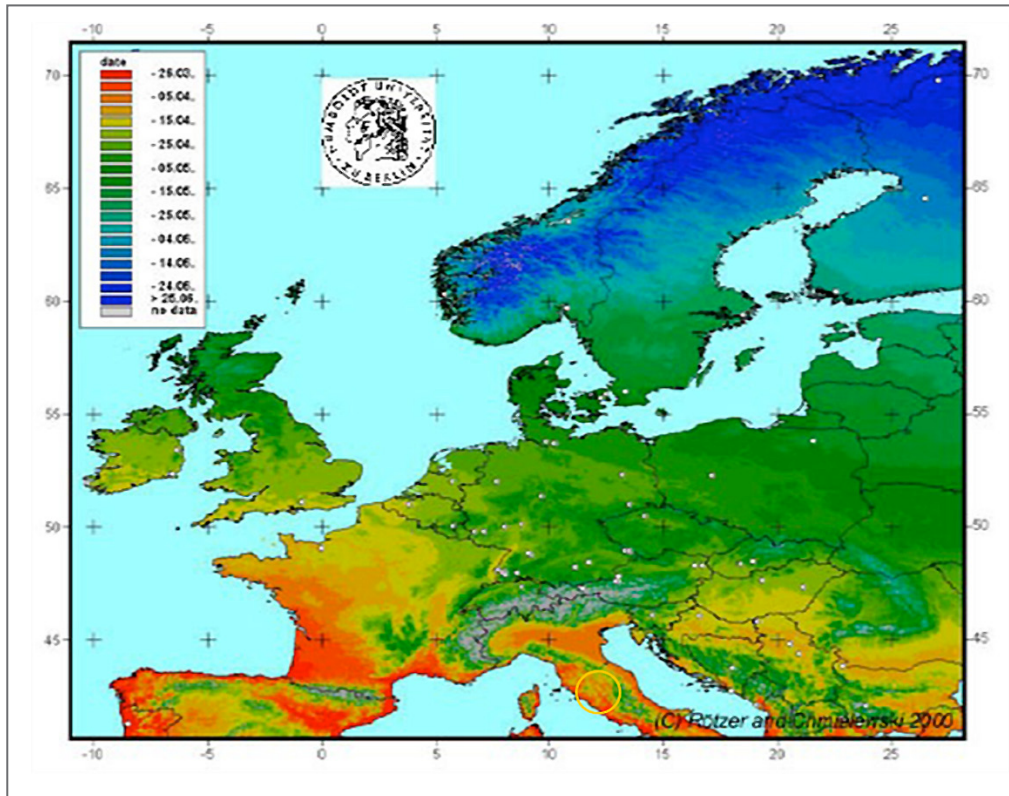
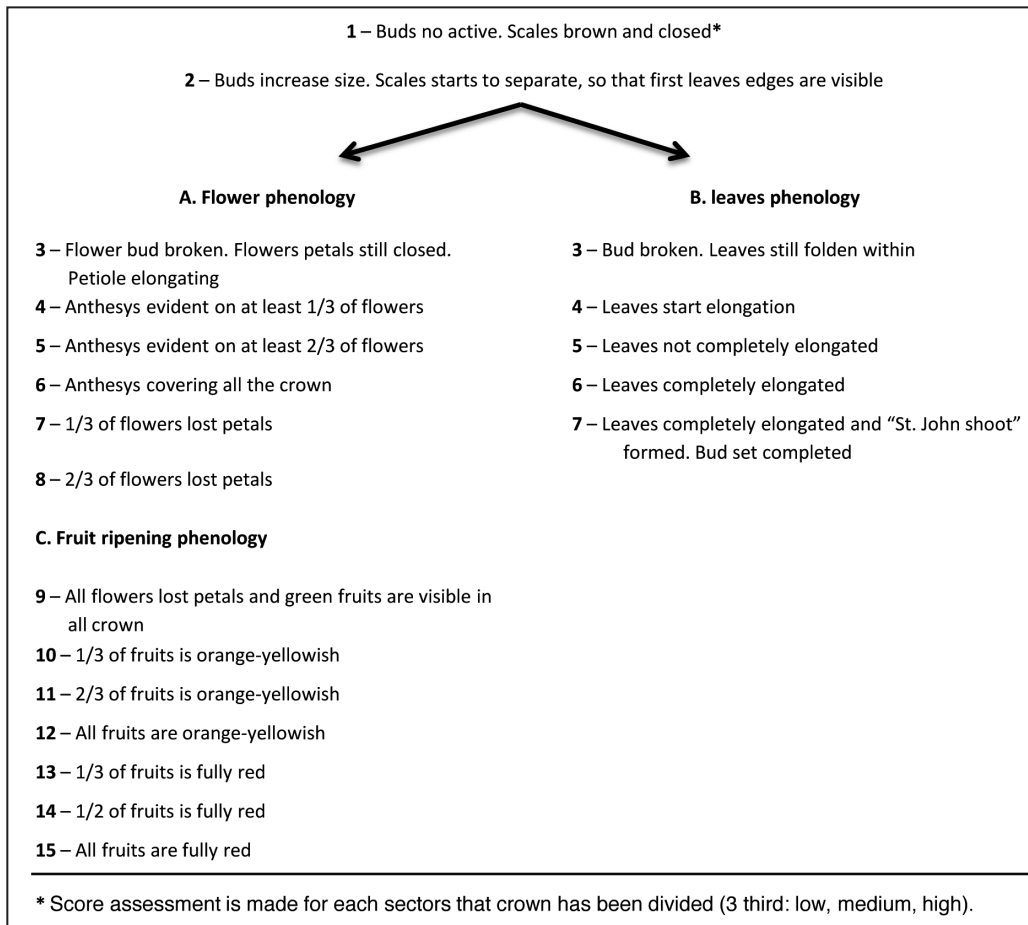


Figure 1. Phenology (*flower bud burst*) map of sweet cherry varieties used in Europe, based on averaged data.

LOGIC SCHEME OF THE METHOD



Scheme 1 - Integrated methods for phenology assessment in *P. avium*.

INTEGRATED SCORING SYSTEM FOR WILD CHERRY (*PRUNUS AVIUM* L.) PHENOLOGY TRAITS

Common phases and scores

- 1 - Buds no active, scales brown and closed.
- 2 - Buds increase size, scales start to separate so that first leaf edges are visible.



1



2

A - Flower phenology



- 3** Flower bud broken, flowers petals still closed, petiole elongating.



- 4** Anthesis evident on at least 1/3 of flowers.



- 5** Anthesis evident on at least 2/3 of flowers.



- 6** Anthesis covering all the crown.



- 7** 1/3 of flower lost petals.



- 8** >2/3 of flowers have lost petals.

B - Leaves phenology



- 3** Bud broken, leaves still folding within.



- 4** Leaves start elongation.



- 5** Leaves not completely elongated.



- 6** Leaves completely elongated.



- 7** Leaves completely developed and in some cases the St. John shoot is formed.

C - Fruit ripening phenology



9

1/1 of cherries is green.



10

< 1/3 of cherries is orange-yellowish



11

<2/3 of cherries are orange-yellowish.



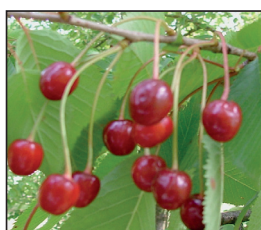
12

2/3 to 1/1 of cherries are orange-yellowish.



13

<1/3 of cherries is fully red.



14

< 2/3 of cherries is fully red.



15

> 2/3 to 1/1 of cherries is fully red (Bordeaux).

Reference genotypes for phenological (population, family, clone) were chosen for their higher h^2G or H^2C values (Table 1).

Table1. Reference genotypes for flowering and phenology.

		Very early flowering	Very late flowering
Wild cherry	Progenies	AN060 and AN103 (BE)	SSG014 and SSG019(BE)
	Clones	BF06, BF03, BF09 (IT)	VC03, VF01, VLN06 (IT)

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Treebreedex

TREEBREEDEX RESEARCH INFRASTRUCTURE NETWORK 2006 -2011

(European project CT2006-026076)

ADAPTIVE TRAITS: OTHER TRAITS



Polycyclic plantation for biomass and higher quality woody production in the Po Valley (Lodi, Milan - Italy. Authors: F. Pelleri, C. Bidini; CRA-SEL)

Traits: **Assessment of frost hardiness**

Conifers/Broadleaves

Date: March 2011

ASSESSMENT OF FROST HARDINESS OF YOUNG TREES

DEFINITION

Frost hardiness is the ability of trees to survive the effects of temperatures below the freezing point. More specifically, it is the ability of trees to tolerate frost of different levels.

The development of frost hardiness within trees is influenced by several exogenous factors *e. g.* course of temperature and day length during the year. It is also affected by endogenous factors such as the development rhythm of the tree, the accumulation of plant contents or the speed of metabolism. Therefore, frost hardiness cannot be considered as a fixed condition lasting constantly for a long period, but it should be understood as a process which is subject to permanent change.

Different phases of frost hardiness are considered (Scheumann, 1964; 1968):

- the ability of plants for hardening (early frost hardiness);
- the degree of hardening (winter frost hardiness);
- the response to significant changes of temperature during the winter period (stability of frost hardiness);
- the beginning of dehardening (late frost hardiness);
- the ability to regenerate damages caused by frost.

Since the natural conditions may change in an unpredictable way, frost hardiness should be assessed under controlled conditions in climate chambers. Therefore, the plant material should be tested: I. immediately after the vegetation period prior to the first frosts; II. during the winter period; III. finally at the end of the winter season.

In addition, assessment of the annual growth rhythm in field trials, such as bud flush and bud set, may provide valuable information for ranking provenances, families or clones according to their frost hardiness in different periods of the growth season.

ASSESSMENT OF FROST HARDINESS

Plant material

To avoid the use of material which is already damaged, an equivalent number of plants per genetic unit should be assessed on damages before the tests.

The plant material to be used for the assessment of frost hardiness should be cultivated for a longer period under more or less homogenous laboratory or field conditions.

Sampling

Samples

Frost tests are commonly carried out with young plants in pots or containers or, if the trees are older and larger, with parts of plants *e.g.* lignified shoots of the current year.

Sampling size

The sample size depends mainly on the genetic background of the plant material. Using clone material will require a smaller number of samples compared to seedlings (half- and full-sib progenies) as the variation to be expected

may be smaller in the former case. Therefore, the number of sampled plants will increase from 3 - 5 ramets per clone to 15 - 30 seedlings per progeny.

The number of samples to be included in the experiments also depends on i. the statistical analysis of the data (Johnsen, 1989b) and ii. on the number of test levels (cf. infra) and the number of replicates per test level.

Sampling procedure

The sampling procedure depends on the number of plants available. If there is only a limited number of plants available, all plants are sampled. If there is a large number of plants available, plants can be sampled randomly (e.g. every third plant) or selectively according to a range of diameter (root collar diameter, DBH). The latter approach requires the measurement of the selection parameters.

If parts of plants of larger trees are used for freezing experiments (e.g. twigs), the twigs should be taken from branches at the same height of the tree e.g. at 1.3 m. The twigs should have a length of 15 to 20 cm. After cutting, the twigs should be placed immediately in plastic bags, labelled and put into cool boxes. After transport, the material should be put into humid sand and kept already under pre-freezing temperature conditions until testing.

Time of sampling

Since frost hardiness is to be considered as a dynamic process, the time of sampling is crucial for the assessment of the different types of frost hardiness:

- early frost hardiness: immediately after the vegetation period before the first frosts
- winter frost hardiness: during the winter period;
- late frost hardiness: at the end of the winter season.

If the stability of frost hardiness during the winter season is to be assessed, the first and the last sampling should be done at the beginning and at the end of the winter season respectively. During the winter season, a more or less frequent sampling during the winter season is necessary depending on the course of temperature.

Freezing procedure

Frost killing point

The frost killing point LT_{50} is the temperature at which 50 % of the plant material dies due to freezing injury.

Depending on the kind of frost hardiness investigated, different test temperature are required. In order to facilitate the estimation of the "frost killing point" (LT_{50}) a minimum of five test levels with a fixed distance between the levels is recommended (Table 1).

Independently of the plant material (buds, needles, plants) and the assessment methods, the data achieved can be used to estimate the temperature damaging 50 % (LT_{50}) of the material in question by arithmetical interpolation as described by Weber (1986) or by statistical methods described for example by O'Reilly *et al.* (2000).

Freezing procedure

The freezing procedure is divided into five steps, the pre freezing period, the constant cooling period, the test period, the constant thawing period and the post thawing period.

Table 1. Freezing test levels for the assessment of frost hardiness.

Purpose	I	II	III	IV	V
Early frost hardiness	-4.0°C	-6.0°C	-8.0°C	-10°C	-12°C
Winter frost hardiness	-8.0°C	-14.0°C	-20.0°C	-26.0°C	-32.0°C
Late frost hardiness	-4.0°C	-6.0°C	-8.0°C	-10°C	-12°C

Assessment of frost hardiness of seedlings and young plants with instant freezing

After pre-treatment, the plant material is cooled down straight to the test temperature. After freezing the material for 2 hours, the material is thawed back straight to 2 °C for post freezing treatment. After keeping the material at this temperature for to 4 hours, the tissues are transferred to glasshouse, laboratory or nursery facilities for cultivation (Table 2).

Table 2. Standardized test design to be used in instant freezing tests.

Step	Duration and conditions
Pre freezing	4 hours at +2 °C
Cooling to test temperature	4 hours
Freezing at test temperature	2 hours
Thawing from test temperature	4 hours
Post freezing	4 hours at +2 °C

Assessment of frost hardiness of seedlings and young plants with constant freezing

The temperature in the climate chamber is constantly decreased to the test temperature with a fixed rate of -6 K per hour. After 4 hours under constant freezing conditions, the temperature is increased again with a fixed rate of +6 K per hour until a temperature of +2 °C is reached. After keeping the material at this temperature for 3 hours, the tissues are transferred to glasshouse, laboratory or nursery facilities for cultivation (Table 3).

This procedure requires the availability of a climate chamber with flexible temperature processing.

Table 3. Standardized test design to be used in constant freezing tests.

Step	Duration and conditions
Pre freezing	3 hours at +2 °C
Cooling to test temperature	-6 K per hour
Freezing at test temperature	4 hours
Thawing from test temperature	+6 K per hour
Post freezing	3 hours at +2 °C

Cultivation of tested material

After the freezing procedure, the plant material should be transferred immediately to the cultivation facilities.

The material is cultivated in moderate conditions and with sufficient water supply. As cultivation media sterilized sand is recommended. To keep the moisture content of the air at a high level, the tested material can be covered by plastic. The place of cultivation should be shaded with 14 hours of light at minimum. The temperature should not exceed +20 °C. After 20 days of cultivation, damages are assessed.

Assessment of damages and injuries

Damages and injuries of needles

The discolouration of the needles after freezing is assessed by a multi-point scale (Table 4).

Table 4. Multi-point scales of different accuracy for the assessment of needle damages caused by frost.

Level of accuracy	Stages of discolouration		
	Undamaged	Intermediately damaged	Totally damaged
Overview	1 = No discolouration	2 = up to 33 % discoloured needles 3 = up to 66 % discoloured needles 4 = up to 99 % discoloured needles	5 = All needles discoloured
Precise	1 = No discolouration	2 = up to 20 % discoloured needles 3 = up to 40 % discoloured needles 4 = up to 60 % discoloured needles 5 = up to 80 % discoloured needles 6 = up to 99 % discoloured needles	7 = All needles discoloured
Very precise	1 = No discolouration	2 = up to 10 % discoloured needles 3 = up to 20 % discoloured needles 4 = up to 30 % discoloured needles 5 = up to 40 % discoloured needles 6 = up to 50 % discoloured needles 7 = up to 60 % discoloured needles 8 = up to 70 % discoloured needles 9 = up to 80 % discoloured needles 10 = up to 99 % discoloured needles	11 = All needles discoloured

Damages and injuries of buds

If only twigs or shoots are considered, the apical bud and the lateral buds are assessed using a multi-point scale (Table 5)

Table 5. Multi-point scale for the assessment of bud damages using with three buds at the top of the twig as example.

Scale	Level of damage
1	No bud damaged
2	One bud damaged
3	Two buds damaged
4	All buds damaged

The level of damage can be assessed in two different ways:

1. If seedlings or plants are used without further cultivation, the damage of buds caused by frost is assessed by a longitudinal cut through the apical cone of the buds. Undamaged apical cones are light-green, damaged are brownish or black-brown of colour.
2. If potted or containerized seedlings or plants are used and cultivated further on after the freezing, damages and injuries of buds can also be assessed at the beginning of the vegetation period by the observation of flushing and the survival rate. The scoring scheme for the assessment of the different flushing stages should follow the common protocols for the assessment of phenological traits of the respective species as described by TREEBREEDEX

The total number of damaged buds is counted and the percentage seedling/plant is calculated.

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Traits: **Biomass assessment**

Conifers/Broadleaves

Date: March 2011

PROTOCOL FOR ASSESSMENT OF BIOMASS

DEFINITION

Biomass is the total weight of organic material in a particular sample, region, trophic level, etc. at a certain date (Schuett *et al.*, 1992; King and Stansfield, 1997). Biomass can be expressed as fresh or dry weight. In a forestry context, biomass is often used as a synonym for the organic substances produced by plants, i.e. phytomass.

The protocol presented serves the assessment of dry biomass of whole trees, with the exception of roots and leaves, in forest stands including short rotation plantations up to 20 years. Thereby, the productivity of clones, full-sib and half-sib progenies and provenances can be mutually compared and/or related to varying environmental conditions.

ASSESSMENT

Methodology

Biomass is assessed using the “regression-method”, based on the development of allometric functions which describe the relation between the weight of trees and easy to measure parameters (Verwijst and Telenius, 1999; Roehle *et al.*, 2006). The advantage of biomass functions is that they require only a few stand parameters as input variables which can be easily recorded in the field (Roehle *et al.*, 2006).

Sampling

Depending on the age of the plant material, a sample size of 50 to 200 trees per genetic unit is recommended bearing in mind that:

- less trees are required with increasing age of the plants;
- the number of sample trees increases when passing from clones to provenances as genetic units (Figure 1).

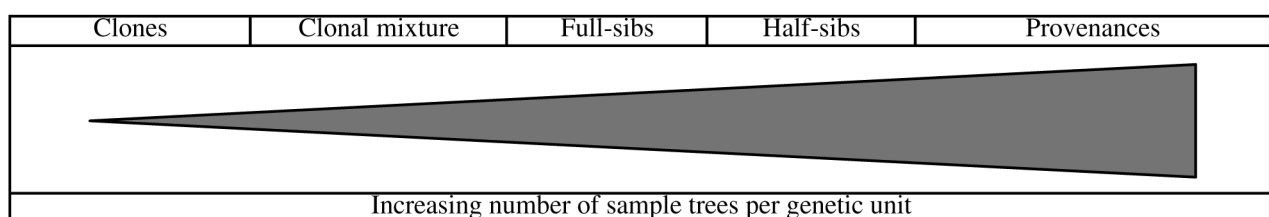


Figure 1. Number of sample trees in relation to the type of genetic units to be investigated.

If only a limited number of trees are available, a sample size down to 20 may be sufficient. The area of the sample plot depends on the number of sample trees.

The diameter at breast height (DBH) and subsequently the diameter range of all trees within the sample plot is recorded with a precision of 1 mm.

Next, ten sample trees are selected covering the whole DBH range. In case of trees with heavy branches and/or many branches, the number of trees is raised to 15. The DBH of these “biomass-trees” is measured once again with a precision of 1 mm.

Assessment of fresh and dry weight

After harvest, the fresh weight of the sample trees (FW_{st}) is to be determined.

For the assessment of dry weight it is necessary to take a biomass sample. If trees are smaller than 5 m, the whole tree can be used as sample. If trees are taller than 5 m a biomass sample is to be taken representing about 10 % of the whole tree. A proportion of the branches in relation to the whole tree should be included in the sample (Figure 2).

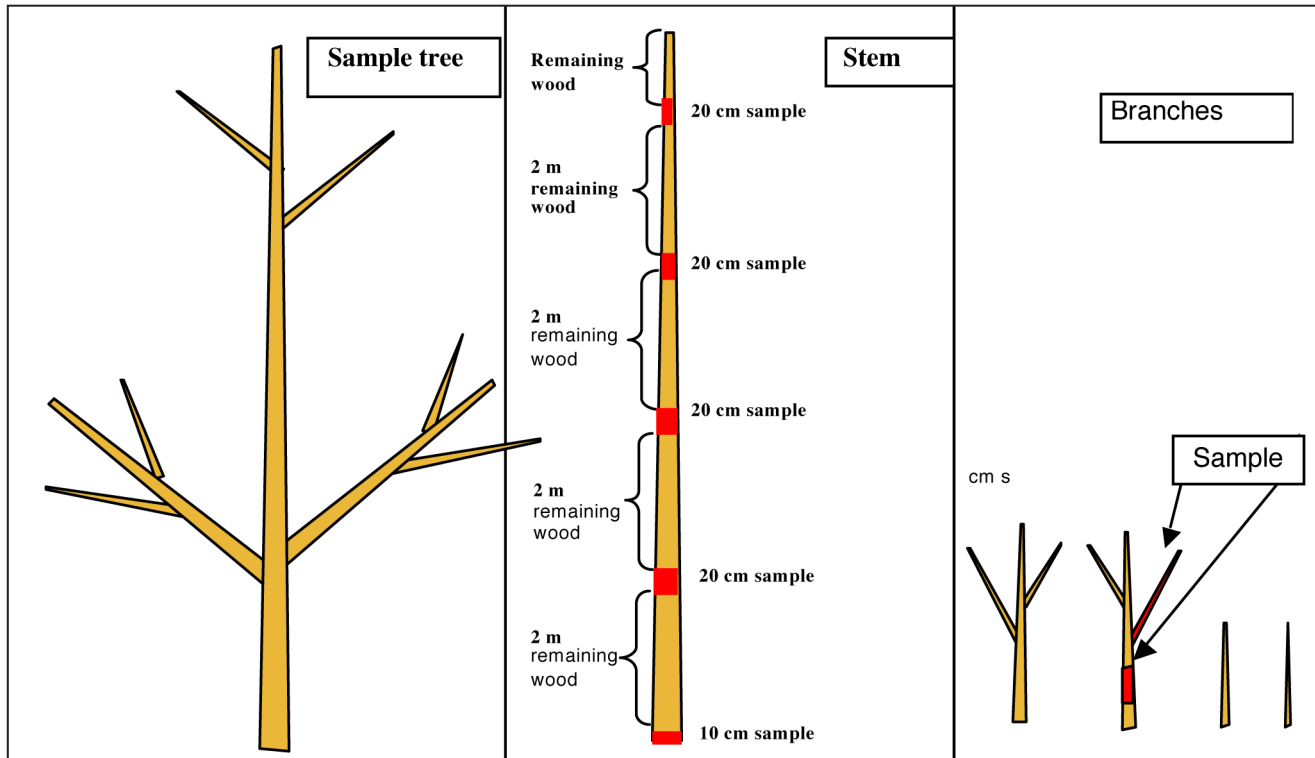


Figure 2. Biomass sampling for trees higher than 5 m (up to 20 m).

Biomass samples (FW_{bs}) can be weighed *in situ* or the same day in the laboratory after packing them in watertight plastic bags.

Fresh weight is to be determined using scales with a precision of 1 %.

Prior to assessment of dry weight, the samples are to chip. Wood chips are to be dried at 104 °C up to constant weight (about 48 h). Dry biomass (DW_{bs}) is to be determined with a precision of 1 %.

Based on the dry weight of the biomass samples (DW_{bs}), the total dry weight of the sample trees (DW_{st}) can be calculated (Table 1).

Table 1. Calculation of the total dry weight of the sample tree.

$DW_{(st)} = DW_{(bs)} * FW_{(st)} / FW_{(bs)}$
$DW_{(st)}$ = Dry weight of sample tree
$DW_{(bs)}$ = Dry weight of biomass sample dried in the laboratory
$FW_{(st)}$ = Fresh weight of sample tree
$FW_{(bs)}$ = Fresh weight of biomass sample dried in the laboratory

Establishment of an allometric function

Based on the paired values of DBH and total dry weight of each sample tree, a regression function is calculated, i.e. the coefficient a_0 and a_1 are determined (e.g. using MS Excel) (Table 2).

Table 2. Allometric biomass function

$DW_{(sts)} = a_0 * DBH_{(sts)}^{a_1}$
$DW_{(sts)}$ = Dry weight of sample tree (= biomass) a_0 & a_1 = Coefficients of the regression function $DBH_{(sts)}$ = Diameter at breast height of sample tree

Assessment of the total biomass in the sample plot

Once the biomass function has been established, the biomass of all trees in the sample plot can be determined by entering their DBH in the function. The biomass per unit area can be calculated based on the surface of the sample plot (Table 3).

Table 3. Assessment of biomass per unit area.

$BM = BM_{(sp)} / A_{(sp)}$
BM = Biomass/Area (kg/ha) $BM_{(sp)}$ = Biomass of the trees of the sample plot (kg) $A_{(sp)}$ = Area of the sample plot (ha)

Development of yield estimations

Biomass functions can be developed for different conditions, i.e. site characteristics, number of trees/ha, etc. First approaches have been done for poplar (Hartmann, 2010).

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