



REsource INFrastructures for monitoring, adapting and protecting European Atlantic FORests under Changing climatE



REINFFORCE ARBORETUM FIELD MANUAL

Version 20

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Introduction

This manual describes methods for monitoring growth and health of the REINFFORCE network sites. Monitoring is a critical element of sustainable forest management. The protocols here are intended for use in all the arboreta of the project and carried out by the project's partners. The intent of developing a manual is to standardize data collection methods and tools so that data can be more easily shared, compared and analysed amongst the different partners. The purpose of this document is to provide detailed descriptions of the field data collection methods for the following monitoring protocols:

- Site description
 - Site definition
 - Soil analysis
 - Understorey description
- Stand description
 - Tree measurements (Growth)
 - Biotic and abiotic damage assessment (Health)
 - Phenology
- Site management

The manual is organized into the sections that correspond to the assessment protocols listed above and the site management. Other than the monitoring site establishment protocol, each protocol has a corresponding field diary.

The data collected will have to be uploaded on the REINFFORCE website to guarantee the accessibility to the network. A table with all the variables can be found on the following link: http://www.iefc.net/bdd/montre_structure_base.php?database=treedata

1. Sampling strategy

A. Data collection frequency

During the REINFFORCE week it was decided to have two levels of monitoring. The standard level will be applied to all the REINFFORCE sites. All the partners commit in collecting all the mandatory data, they can collect optional data if they have the opportunity.

All the data must be collected according to this protocol. This will ensure the consistency among the sites.

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weather	x	x	x	x	x	x	х	х	x	x	x	x :	x	x :	x	x	x	x	x	x	x	x x	(x	x	x	х	х	x	x	х	x	$\langle \rangle$	$\langle \rangle$	$\langle \rangle$	x	x	x	x	x	x	x	x	×	x	x	x	x	x	x	x	x	x	x
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Table 1: protocols application during the first 15 years of the arboreta after 2011 for all sites, x: manadatory; o: optional

The sites listed in the table 2, will be intensively monitored collecting all the optional data.

PARTNER	Number of Site per partner	SITE NAME
FR	1/3	Westonbirt
CNPF - IDF	0/9	
INRA	2/4	Pierroton
GAN	1/2	Etxauri
NEIKER	1/5	Basque Country
FGUVA	1/5	Castilla y Leon
CIF Lourizan	1/3	Galicia
ISA	1/3 or 2/3	Portugal

Table 2: intensively monitored sites

B. Sampling on the field

• The growth protocol will be applied starting from 2011 and all the assessment will be done once per year, in the first 5 years as an intensive monitoring. It must be done when tree growth is stopped (winter time: between November and March according to the region). 100% of the trees are measured. Each time growth is monitored, understorey is also described.

• The health protocol will be applied every 2 years, in the same year of the growth protocol. They have to be done in the same vegetation period. It is important to make the health assessment at the end of (or during) the summer before the leaf falling. The data collected will be linked to the dendrometric data acquired on the next winter.

- On 6 trees / Genetic Unit

- 2 Genetic Units / species, or 6 Genetic Units for the 4 species with 3 replicates
- 32 species
- (28 x 2 x 6) + (4 x 6 x 6) = 480 trees / arboretum
- Sequential sampling: spend the same type, looking at presence/absence, e.g. 5'

• The phenology protocol will be mainly applied at the intensively monitored sites, so the assessment will be done every year but only after the 3rd year from the installation. The optimum period for the phenology monitoring is from the beginning of the growing season (February, depending on the local climate) up to the end of the growing season (November, depending on the local climate), and it has to be applied to all the trees. All the monitored sites must be controlled on the 1st Monday of each month.

All the collected data will be stored in the data-base of the project. The data uploading is under responsibility of each partner (of consortium agreement).

2. Site description

2.1. Site definition

Purpose: to provide the general description of the location and characteristics of each monitoring site location.

C. Site number

A number of the site will be assigned by the coordinator on the base of the latitudinal distribution. *Variable: ID_SITE*

D. Field crew and date

The month/day/year on which the data on the form is gathered in the field will be recorded, along with the names of the field crew.

E. Latitude, longitude and altitude

The latitude, longitude and altitude of the centre of the site will be recorded setting the UTM zone.

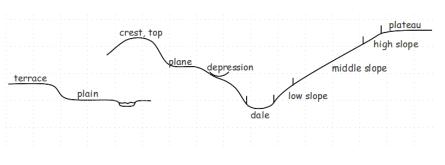
F. Fencing of the site

Description of type and height, and modality of fencing. *Variable:* for site fencing: *Fs_m*; for individual fencing: *Fi_m*

G. Topography

> Site position

The site position describes the relative position of the sampling site within a catchment area



Site position	Code
Plateau	1
high slope	2
middle slope	3
low slope	4
Dale	5
Depression	6
Plane	7
crest,top	8
Plain	9
Terrace	10

Illustration 1: site position. Figure adapted from P.Gonin, 2010

Table 3: codes for site position categories

Variable: SP [Code]

Aspect and slope

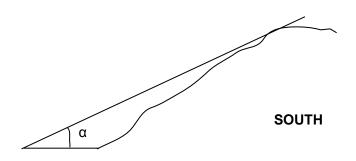
• Aspect: it measures the orientation of a slope and indicates whether the slope exposed to the north, south, east, or west, or any point between. The aspect will have one of the following categories: North, North-East, East, South-East, South, South-West, West, North-est, or flat.

Orientation	Ν	NE	E	SE	S	SW	W	NW	FLAT
Code	1	2	3	4	5	6	7	8	9

Table 4: codes for the aspect's categories

Variable: O [Code]

- **Slope:** the percent slope gradient of the land at the site will be measured using a clinometer, following the line of the maximum slope. *Variable: SLOPE [%]*
- > Shadowing



Shadowing is the effect of the hill covering the site from the South influence. It is the angle (α) between the horizontal plane (the site location) and the highest point of the hill. *Variable:* SH [°]

H. Soil profile description and soil type: optional

There are several internationally accepted systems of soil description and soil classification e.g. the FAO-UNESCO *Legend for the soil map of the world* (FAO-UNESCO, 1974, 1988), Soil Taxonomy (USDA, Soil Survey Staff, 1975, 1999) or Soil Atlas of Europe (EC, 2005). These systems are based on the internationally accepted Guidelines for Soil Description (FAO, 1990) and all of them follow a very similar structure:

- I. General site description
- II. Description of soil forming factors
- III. Horizon designation and soil classification

RECOMENDATION: for this classification it is advisable to refer to the most usual system applied at local level. The name of soil types will be standardised for the data base application.

The **World Reference Base for Soil Resources (WRB)** will be applied for soil classification encoding in the online database.

A humus type classification will be also added to the site definition (MOR, MODER, MULL).

RECOMENDATION: for this classification it is advisable to refer to the most usual system applied at local level. The name of bedrock types will be standardised for the data base application using the geographical Database of Eurasia.

	aphical Database of Eurasia.		
	Code for dom inant parent material		Code for dom inant parent material
	consolidated clastic sedimentary rocks		acid regionalmetamorphic rocks
2	conglom erate		m ic a s c h is t
3	breccia	44	g n e is s
4	psammite or arenite	4 5	g r e e n s c h is t
5	s a n d s to n e	4 6	s e r p e n tin ite
6	calcareous sandstone	4 7	calcareous regional metamorphic rocks
7	ferruginous sandstone	4 8	tectogenetic metamorphism rock or cataclasmic metamorphism
8	clayey sandstone	4 9	unconsolidated deposits (alluvium, w eathering residuum and slope deposits)
9	quartzitic sandstone/orthoquartzite	50	marine and estuarine sands
	arkose	51	holocene coastal sand with shells marine and estuarine clays and silts
11	pelite, lutite or argilite	52	pre quaternary clay and silt
12	c la y s to n e /m u d s to n e	53	tertiary clay
13	s ilts to n e	54	quaternary clay and silt
14	facies bound rock	5 5	fluvial sands and gravels
15	flysch	56	river terrace sand or gravel
16	m o la s s e	57	river terrace sand
17	calcareous rocks	58	fluvial clays, silts and loams
18	lim eston e	59	terrace clay and silt
19	hard limestone	6 0	lake deposits
20	soft lim eston e		residual and redeposited loams from silicate rocks
21	marly limestone	62	residual loam
22	chalky limestone	63	stony loam
23	detrital limestone	64	clayey loam
24	d o lo m ite	6 5	residual and redeposited clays from calcareous rocks
25	mari	6 6	d u a l c la y
26	gypsiferous marl	67	clay w ith flints
27	ch a lk	68	ferruginous residual clay
28	acid to intermediate plutonic rocks	69	calcareous clay
29	g r a n ite	70	stony clay
30	d io rite	71	slope deposits
31	basic plutonic rocks	72	colluvial deposits
32	gabbro	73	un con solidated glacial deposits/glacial drift
33	acid to intermediate volcanic rocks	74	morainic deposits
34	rh y o lite	75	outw ash sand, glacial sand
	andesite	76	eolian deposits
36	basic to ultrabasic volcanic rocks	77	10 e s s
37	b a s a lt	78	loamy loess
38	pyroclastic rocks (tephra)	79	sandy loess
39	tu ff/tu ff stone	80	eolian sands
4 0	(m e ta) s h a le /a rg ilite	81	dune sand
41	s la te	8 2	coversand
		83	organic materials
		84	peat (mires)
Tab	le 13. Geographical Database of Eurasia	85	slime and ooze deposits

In colour: dominant parent material.

J. Plot characteristics

Initial tree number: Number of trees planted at the beginning of the trial.

Structure of the plot: Explain how trees have been spaced; using a square, rectangle, quinconce, etc.; design. The choice list can be extended on demand.

Spacing: distance between trees (3 meters by 3 meters, 2,5 meters by 3,5, etc.). The choice list can be updated on demand.

2.2. Soil analysis

K. Sample collection before soil preparation

Organic horizon (litter):

• estimation of the litter covering surface respect to the total area of the site

• the living matter (herbaceous) is not included in the sample. It will be counted in the understorey

- · some variables have to be measured
- total depth of the litter and relative importance of the layer
- Variable: LayerL [cm], LayerF [cm], LayerH [cm];
- if possible, describe the different layers of the sample

Intact litter/dead surface cover	leaves and/or intact acicular on the ground	layer "L"
Litter/fragmented cover	leaves and/or fragmented acicular, where it is still possible to identify the origin of the organic matter that forms it	layer "F"
Humus	horizon that is in a humidification stage, placed right under the fragmented dead leaves, where it is impossible to identify the origin of the organic matter that forms it	layer "H"

Table 5: Litter composition

L. Sample collection after soil preparation

Using the 60 cm long auger, soil samples should be taken in a cross defined by the North to South axis and the East to West axis in a regular grid of points. The centre of the cross should be placed at the centre of the arboretum according to the figure shown below (Illustration 2). This way, 16 points in each arboretum would be sampled.

In each of these points, we would take 2 samples of soil, the first at a depth of 0-30 cm, and the second at a depth of 30-60 cm.

In addition, at the end points of the cross and in the centre of it, it is recommended to use the 1 m long auger to measure effective depth. Another sample from the depth of 0-30 cm and from the depth of 30-60cm will then be taken.

The 21 samples from the 0-30 cm would be mixed in a plastic bag previously identified. The same procedure would be followed with samples taken from the 30-60 cm depth.

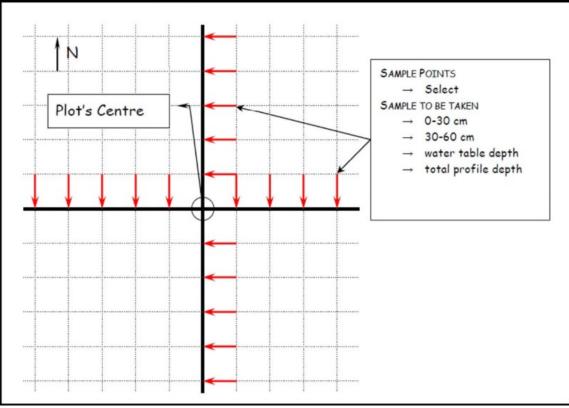


Illustration 2: Location of the sample points for soil characterization at the REINFFORCE arboreta. (Figure adapted from Condé and Orazio 2005).

Other aspects to take into account:

- remove the forest floor before taking the soil sample with a soil auger.
- in case there is an obstacle to the sample extraction, it will be taken note of the total soil depth.
- if there is no problem on the samples extraction, it is advisable to take note of the total profile's depth on one of the samples' points, randomly selected
- it is important to take note of the water table depth to establish a possible relation between this factor and the sanitary status (e.g., die-back)

M. Mandatory analysis

- Soil pH in water (1:5 soil:water). If pH > 8, measure pH in CaCl2 and if pH-CaCl2 > 6, measure the carbonate content. Variable: S00-30-pH, S30-60-pH
- Soil texture. Variable: S00-30-Sand %, S30-60-Sand %, S00-30-Silt %, S30-60-Silt %, S00-30-Clay %, S30-60-Clay %.
- Soil organic carbon. Variable: S00-30-Corg (mg kg-1), S30-60-Corg (mg kg-1)

N. Optional analysis

- Total carbon, total nitrogen (units: mg kg-1). Variable: S00-30-TC (mg kg-1), S30-60-TC (mg kg-1); S00-30-TN (mg kg-1), S30-60-TN (mg kg-1)
- Carbonate content (units: g kg-1). Variable: S00-30-C (mg kg-1), S30-60-C (mg kg-1)
- Available P (units: mg kg-1). Variable: S00-30-P (mg kg-1), S30-60-P (mg kg-1)
- Exchangeable cations (Ca, Mg, K) (units: meq/100 g) and cation exchange capacity (CEC) (units: cmol+ kg-1). Variable: S00-30-Exc (meq/100 g), S30-60-Exc (meq/100 g); S00-30-CEC (cmol+ kg-1), S30-60-CEC (cmol+ kg-1)
- Water content at field capacity (-33 KPa) and wilting point (-1500KPa) (units: %). Variable: S00-30-WFC %, S30-60-WFC %; S00-30-WP %, S30-60-WP %
- Bulk density (units: g cm-3). Variable: S00-30-BD (g cm-3), S30-60-BD (g cm-3)

O. Soil analysis procedure guide:

- ISO 10390:2005 Soil quality Determination of pH
- ISO 10693: 1994 Soil quality Determination of carbonate content- Volumetric method. ISO 14235:1998 Soil quality Determination of organic carbon by
- sulfochromic oxidation
- ISO 13320:2009 Particle size analysis Laser diffraction methods
- ISO 11277:1998 Soil quality Determination of particle size distribution in mineral soil material-Method by sieving and sedimentation
- ISO 13878:1998 Soil quality Determination of organic and total
- carbon after dry combustion (elementary analysis);
- ISO 10694:1995 Soil quality Determination of total nitrogen content by dry combustion (elemental analysis)
- ISO 23470:2007 Soil quality Determination of effective cation exchange capacity (eCEC) and exchangable cations using hexaamminecobalt trichloride solution.
- ISO 11260:1994 Soil quality Determination of effective cation exchange capacity (eCEC) and base saturation level using barium chloride solution.
- ISO 14254:1994 Soil quality Determination of exchangeable acidity in barium
- ISO 11263:1994 Soil quality -- Determination of phosphorus -- Spectrometric determination of phosphorus soluble in sodium hydrogen carbonate solution
- ISO 11272:1993. Soil quality- Determination of dry bulk density.
- Water content at field capacity (-33 KPa) and wilting point (-1500 KPa) using a pressure plate extractor. http://www.soilmoisture.com/PDF%20Files/81500.pdf ç

P. Water holding capacity of soil

According to FAO and USDA the water holding capacity of soil is a function of the available water content, rock fragment content (>2 mm), and soil depth. There are four important levels of soil moisture content that reflect the availability of water in the soil.

When a soil is saturated, the soil pores are filled with water and nearly all of the air in the soil has been displaced by water. The water held in the soil between saturation and field capacity is gravitational water. Frequently, gravitational water will take a few days to drain through the soil profile and some can be absorbed by roots of plants.

Field capacity is defined as the level of soil moisture left in the soil after drainage of the gravitational water. The soil is considered to be at field capacity when the water potential in the soil is at -33 kPa. Water held between field capacity and the wilting point is available for plant use.

The wilting point is defined as the soil moisture content where most plants cannot exert enough force to remove water from small pores in the soil. The permanent wilting point is the water content of the soil at -1.5 MPa water potential. Most crops will be permanently damaged if the soil moisture content is allowed to reach the wilting point. In many cases, yield reductions may occur long before this point is reached. Capillary water held in the soil beyond the wilting point can only be removed by evaporation. When soil is dried in an oven, nearly all water is removed. "Oven dry" moisture content is used to provide a reference for measuring the other three soil moisture contents.

A pressure plate extractor is used to determine the water retention of soil samples at -33kPa and the water potential at -1500KPa and it will be estimated from the soil samples.

LEVELS OF SOIL MOISTURE	Moisture content (%)
FIELD CAPACITY	25%
WILTING POINT	16% Available water (%)

Table 6: soil moisture classification

Variable: SM_FC (%), SM_WP (%)

2.3. Understorey description

It consists on inventorying before or just after planting the percentage of cover (% of the soil cover by vegetation, if the sum for layer exceed 100% please comment) and the Total height of the dominating species (the main species) composing the understorey.

Understorey	%	H(cm)	MAIN SPECIES (Latin name)
TREES			
SHRUBS			
FERNS			
MOSS			
GRASS			
OTHER			

Table 7: understorey data record (by site)

The table can be filled for the whole surface or homogeneous parts.

Variable:

UNDERSTOREY_T (%), UNDERSTOREY_T_ (cm), UNDERSTOREY_T_main_species; UNDERSTOREY_S (%), UNDERSTOREY_S_ (cm), UNDERSTOREY_S_main_species; UNDERSTOREY_F (%), UNDERSTOREY_F_ (cm), UNDERSTOREY_F_main_species; UNDERSTOREY_M (%), UNDERSTOREY_M_ (cm), UNDERSTOREY_M_main_species; UNDERSTOREY_G (%), UNDERSTOREY_G_ (cm), UNDERSTOREY_G_main_species; UNDERSTOREY_O (%), UNDERSTOREY_O_ (cm), UNDERSTOREY_O_main_species;

3. Weather parameters

PARAMETERS	Variable	Frequency
Max-min daily Temperature	T_max / T_min (°C)	Daily
Daily Precipitation	P_mm	Daily
Relative humidity (day/night)	Rhmin- RHmax (%)	Day/Night
Solar radiation	I (W/m²)	Daily
Soil moisture outside stand 50cm?(optional)	SM50 (%)	Daily
Evapotranspiration	ET (mm)	Daily
Evapotranspiration computing methods	ET_computing_methods	Punctual
Dew point	DPmin (decimal) / DPavg (decimal)	Daily
Number of hours above 5°C	NB_hours_above_5	Daily

Table 8: weather parameter record

4. Stand description

4.1. Tree measurements (Growth)

Q. Tree Numbering

A label will be affixed to each tree on which is printed the species Latin name, provenance and code. Additional stems will be noted with letters. The order number is assigned according to the path followed for measuring.

SPECIES NAME AND PROVENANCE		ORDER TREE NUMBER	ADDITIONAL STEMS
	CODE	[1-2000]	A, B, C, etc.

Table 9: example of species name and provenance coding

The identification of the tree in the database results as follows:

ARXX-NAME-PROV-CODE

(Example: AR01-ACPS-ALPS-01)

where ARXX is the arboretum number, NAME is the tree species, PROV is the provenance code and CODE is the tree number. The combination NAME-PROV has to be included on the list of Provenances of the project.

In case dying trees are replaced (because they are dead, missing, etc), the new tree will get a new final number. The replaced tree gets the Status "Replaced" (see D - Status) and the new planted tree gets a new code and plantation date (new row).

R. Total Height (H)

The tree's total height (H) will be defined as *the distance from the base of the tree to its top (higher point)*. The higher point of the tree is sometimes difficult to establish due to the leaves.

- > Height \leq 6m: the measurement will be taken with a rod graduated in cm
- > Height > 6m: the measurement will be taken with a hypsometer;
- The measurements will be taken on each tree and only the living part of the tree will be included
- In case there is a slope, we will always measure on the highest area
- In case of forked trees, the tallest part of the tree will be measured;

Variable: H (cm)

S. Circumference

The CBH - Circumference breast height or circumference 1,30 is the circumference of the stem of a tree measured at breast height (1,30m) from the ground. - *note1:* on sloping grounds the measure is taken from the up-hill side.

The most common tool to be used on this kind of measurements is the tape.

> When H>1.5m the measurement will be taken with the tape at the marked height

Variable: CBH (cm)

When H<1.5m (OPTIONAL) the diameter of the root collar will be measured with the calliper. In this case, the diameter (D0) and the height where it was measured (HD0) should be included. Variable: D0 (mm); HD0 (cm)</p>

Before measurements all trees are marked at measurement height with paint.

- Trees with special characteristics
- Forked trees:

when the tree forks at 1.0 to 1.30m each stem will be measured 30 cm above and they will be inventoried as two different trunks (ID_Tree is the same but Tree_data.ID_Trunck is different). By defaut, for normal trees, ID_Trunk=1.

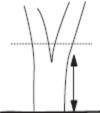


Illustration 3: tree forked at 1.0 to 1.30 m

- Resprouting trees: when the previous stem was broken or dead and a new stem comes, the new stem is given a new ID trunk.
- Trees with protuberances (hypertrophies), branches appearance, and so on:

the defect must not be included in the cubing, following some procedures

b) to carry out the measurement of two circumferences, one above the normal height and another one below, establishing the average measure afterwards

c) to carry out the measurement of only one circumference, placing it a little above or below the normal height, always and when the displacement is not more than 10cm

d) if the defect covers a great part of the trunk (as the case of ice, e.g.), the DBH will be measured including the defect, and it will be taken into account in the case of a probable cubing

e) if the defect's entity does not allow to carry out the DBH measurement, the estimation will be an approximate.

T. Status

A visual analysis of dead/alive/missing/resprouting wood will be done. Variable: [Alive, Dead, Missing, Replaced, Resprouting]

U. Apical dominance (for stem height < 6m)

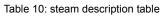
This value corresponds to:

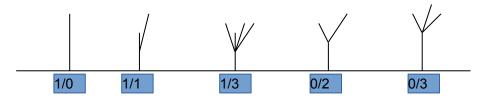
- the number of dominant branches respect to the main axis (variable a) = 1)
- the number of forked branches when the main axis is not present (variable a) = 0).

Note a)	Note b)	Description of the steam
1	0	Apical dominance (axis and one dominant branch)
1	1	Loss of dominance, another dominant branch, but axis preservation
1	3	Loss of dominance, three dominant branch, but axis preservation
0	2	Simple fork
0	3	Three brunches fork

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Variable: Dominance

V. Stem quality description (for stem height > 1,5 m)

Variable	Principle	Sampling	Periodicity	Purpose
Trees straight- ness	One note (from 1 to 4) will be assigned according to the following <u>definitions</u> :	All the stems	Not necessarily at any monitoring, but adapted to the stand growth rate	To appreciate the quality of the global shape of
	1 = straight stem to the whole length			the future timber
	2 = straight stem to the 2/3 of the height, at least			
Variable name: Straightness	3 = straight stem to the 1/3 of height, at least			
Unit: [1;4]	4 = straight stem < 1/3			
Inclination of	One note (from 1 to 3)	All the stems	At any monitoring	To appreciate the
the stem to the vertical	1 ≤20°			quality of the stems
	2 = 20-45°			
Variable name: Inclination	3 ≥ 45°			
Unit: [1;3]				
Branching	One note (from 1 to 3)	All the stems	Not necessarily at any	To appreciate the
	1 = Thin, horizontals and few branches		monitoring, but adapted to the stand growth rate	quality of the stems
	2 = moderately thick, and/ or inclined, and/or moder- ately numerous branches			
Variable name:	3 = thick, and/or highly			
Branching Unit: [1;3]	leant, and/or numerous, and/or diffused whorls branches			
Defected stem	- Height of the defect in meters	All the stems	At any monitoring	To appreciate the quality of the
	- Nature of the defect (K= deep knot, F=fork)			global shape of the future log

4	Defect K (m)	Examples: F6 = forked at 6m K3 = deep knot at 3m		

Table 11: Stem quality description

4.2. Biotic and abiotic damage assessment (Health)

Tree mortality recording after replanting from the second year on, and mortality cause checking by stem debarking and uprooting; send dead seedlings (first 2 years) to the lab for diagnosis.

W. Crown conditions

- 1.1. Crown mortality
 - · Record the proportion of the crown affected branch or shoot mortality
 - Use 5 classes of defoliation: 0-10% (low), 11-25% (ligth), 26%-50% (moderate), > 50% (high), 100%
 - Variable: CrownM [0-4]
- 1.2. Crown defoliation
 - Record the proportion of the crown affected by leaf/needle consumption or damage
 - Use 5 classes of defoliation: 0, 1-10%, 11-50%, > 50%; 100%0-10% (low), 11-25% (ligth), 26%-50% (moderate), > 50% (high), 100%
 - Variable: CrownD [0-4]
- 1.3. Crown discolouration
 - Record the proportion of the crown affected by leaf/needle abnormal colour change (yellow, red, brown)
 - Use 5 classes of discolouration: 0, 1-10%, 11-50%, > 50%, 100% 0-10% (low), 11-25% (ligth), 26%-50% (moderate), > 50% (high), 100%
 - Variable: CrownC [0-4]

The categories have been updated in 2018. To remain consistent in the database they are coded as follow.

	0 .5 1.5 (0-10%)(10-25%)	2.5 (25-50%)	3 (50-100%)	4 (100%)
Updated protocol (FA0 2014)	0% 10% 25%	50%	75% 	100%
Previous protocol:	∎ <u> </u>		3	∎ 4

X. Presence / absence of biotic and abiotic agents

• Tick presence of a particular agent in predefined list of 20 types:

One tree can have several biotic or abiotic variables, you just have to separate them by a comma (,). For example: 3.1-Drought, 3.2-Frost, 3.3-Wind

• Illustrated guide to help identify the agent (ANNEX 2).

9 forest herbivore (insect and mammal) types -Variable: Insect_types
9.1-Chewers
9.2-Gall makers
9.3-Leaf-miners
9.4-Skeletonisers
9.5-Leaf-rollers and tiers
9.6-Sap feeders (honeydew, fumagine)
9.7-Shoot deformation (shoots moth)
9.8-Stem / bark borers (dust, resin, holes)
9.9-Mammal grazer

6 forest disease types - Variable: Disease_types
6.1-Rust
6.2-Mildew
6.3-Leaf Necrosis
6.4-Red Bands (needle cast)
6.5-Canker
6.6-Stem -shoot Necrosis

3 Abiotic types - Variable: Abiotics
3.1-Drought
3.2-Frost
3.3-Wind
3.4-Mechanical damage
3.5-Fire

4.3. Phenology

Y. Introduction to BBCH scale

This protocol will be carried out from the application of the BBCH scale to the phenological stages of the trees.

The BBCH scale is a phenological scale which permits the classification of phenological stages of monocotyledons and dicotyledons, created to standardise the measurement of phenology. The scale is divided into 10 principal stages of development, numbered from zero to nine. Each stage is then subdivided into 10 secondary stages (numbered from zero to nine). A code and description are given to each of these stages, to easily follow vegetation and reproductive development of a plant throughout the season (Meier, 2001).

The scale will be used to classify tree pictures that will be taken in the period from spring to autumn.

Z. Setting up of BBCH scale to REINFFORCE species

This protocol will be finalised only after 2011, once the measurements related to the BBCH method can be applied to the REINFFORCE species after the setting up of the arboreta.

13 species have been selected to start the observation process during the phenological period of 2011/2012. Per each of the listed species (table 12) the partners take the pictures from February to November, notably the starting and the ending moment of the growing season, according to the local climate conditions. It is advisable to take pictures 2 times per week. The BBCH scale for REINFFORCE species is showed in the ANNEX 3.

AA. Updated version of the REINFFORCE phenology protocol made by INRA (coming soon).

INRA has done an excellent work and the protocol has been improved successfully. It will have to be translate from French into English and the Excel tool needs to be more developed and adapted for field work (presented during the virtual meeting in 2016).

REINFFORCE SPECIES – BBCH	PARTNER
Acer pseudoplatanus	FR
Calocedrus decurrens	FR
Cedrus atlantica	FGUVAL
Cedrus libani	FGUVAL
Cupressus sempervirens	ISA
Ceratonia siliqua	ISA
Fagus orientalis	FR
Liquidambar styraciflua	HAZI
Pinus brutia	CIF
Pinus elliottii	CIF
Pinus peuce	FR
Pinus ponderosa	FR
Pinus nigra subsp laricio	HAZI
Pinus nigra subsp salzmanii	HAZI
Pinus pinaster	INRA
Pinus pinea	CIF
Pinus taeda	INRA
Pseudotsuga menziesii	CIF
Quercus rubra and Q. phellos	INRA
Quercus shumardii	FR
Quercus suber	ISA
Robinia pseudoacacia	NEIKER
Sequoia sempervirens	GAN
Eucalyptus nitens (gundal globulus)	ISA
Cunninghamia lanceolata	CIF
Thuja plicata	HAZI+FR

Table 12: species selected for the BBCH scale setting up first attemps before INRA crew test and new protocol development

Variable: BBCH_stage

5. Protocol for site management

All the works related to the site preparation and management must be recorded very precisely. Both for the installation and management it is mandatory to avoid any damage (wound).

BB. Plant Installation

During the year 2010, the partners defined the following rules for arboretum installation:

- A genetic unit is a group of trees corresponding to the same seed lot or clone.
- The amount of trees per genetic unit in each site is 12.
- The spacing can variate between [1000 -1111] tree/ha; there is no mandatory pattern. It can be adapted at the local weed control practices.
- The arboretum sites are supposed to be as homogeneous as possible, all the genetic units have to be grouped in unit as compact as possible (3x4 or 2x6 trees). For pedagogical reasons, easy monitoring and competition limitation, the provenances will be grouped in a nested design:
 - Conifers vs Broadleaved
 - *Pinus* vs other conifers and *Quercus* vs other broadleaved
 - Fast growing species vs slow growing species
 - Some marks should be used to separate the groups.
- As the sites selected for the arboreta installation are supposed to be as homogeneous as possible they are made of one block with all the genetic units except that 4 of them will be replicated three times in all over the site to address site heterogeneity.

The four replicated species are: *Pinus pinaster* (PIPI), *Betula pendula* (BEPE), *Cedrus atlantica* (CEAT), *Quercus robur* (QURO).

- For very heterogeneous sites it is allowed to make two blocks. But this must be exceptional. (Var Block [1;2]).
- The pre-plantation use of herbicides is optional and it will be done during the Spring-Summer period.
- The border effect between trees is not taken into account.
- Where the arboreta are surrounded by other stands it will be installed at a distance equal to the height of the highest stand.

CC. Weather station Installation

One weather station per project site will be installed or an existing one will be used as reference for site climate assessment.

The stations will be installed:

- close enough to sites and under the same topographical conditions as the monitored stands
- in open spaces, for wind speed and direction precision measurements
- on the same type of soil as the monitored stands to measure the corresponding soil humidity
- taking care of leaves removing from the rain gauge
- in a place with a good GSM coverage for the automatic data downloading

DD. Forest Management

- The main management required is weed control and cleaning
- The management of the stands for the first years excludes any kind of human activity: no thinning, no fertilising, etc.

- A corrective pruning will be done for less than 3 years old trees. For Robinia pseudoacacia case: the branches will be pruned but the coppices will not be cut (stem numbers can be recorded in Treedata database) (decision taken during the virtual meeting 2016).
- Shrubs and associated vegetation should be kept below 20 cm during the first 5 years. By the fifth year onwards below 40 cm.
- Individual protection: don't use material that can change the micro-climate conditions.
- No fertilization will be used at the plantation time, but it will consider later on.
- No plastic mulching that can change the micro-climate conditions.
- Any kind of weed control is allowed but AVOIDING chemical contamination of the seedlings.

EE. Risk management

The aim of the arboretum network is to monitor the capacity of various species to survive and growth under a large range of pedo-climatic conditions.

Thus, it is mandatory to support as much as possible the trees during the installation phases to separate the exceptional effects from the regular climatic pressure.

Thus, the partners agreed that the following measure can be undertaken during the first 2 years after the plantation:

- Watering of seedlings, in case of dryness.
- Exceptional conditions (characteristic of the site) may require additional care on the first 2 years.

Two decisions were taken in order to manage the arboreta at the earlier stage of the last plantations in 2013:

- If the mortality is less than 50% of the trees of the same provenance, dying single trees will not have to be replaced with other provenances/species and the holes can be left empty, or replaced with the same provenance.
- If the mortality is more than 50% and there is no way to find the same provenance, the rule is to plant a slow growing species, respecting the nested design of the arboreta (conifers, broadleaves, etc.) It is recommended to coordinate with other partners.

The re-planting period is related to the 2013 plantation results.

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Annexes

ANNEX 1: Field visit short report

ANNEX 2: Guide for biotic and abiotic damage assessment (Health)

ANNEX 3: The BBCH scale for REINFFORCE species

ANNEX 4: Breeding conditions in the nursery

\lefcdata2\efiatlantic\Projects\2009 REINFFORCE\Suivi technique\Unified protocol\ANNEX1(4).pdf

ANNEX 5: Seeds provenances changes

\lefcdata2\efiatlantic\Projects\2009 REINFFORCE\Suivi technique\Unified protocol\ANNEX2(5).pdf

Annex 1: Field visit short report

Site Code:

Site REINFFORCE code:

Visit date:

Report Author:

Visiting team:

Shrub status

Understorey	%	H(cm)	MAIN SPECIES (Latin name)
TREES			
SHRUBS			
FERNS			
MOSS			
GRASS			
OTHER			

Copy this table as many time as needed in case of more than one shrub status in the site.

Work achieved

Time spent:

Observations (problems, fencing status, etc.)

Annex 2: Guide for biotic and abiotic damage assessment

(Health)

For an easier identification of the biotic and abiotic damages, a description of the agents and some images of the damages are provided. The nomenclature and order from chapter 4.2.B. Presence/Absence of biotic and abiotic agents will be followed.

9 forest herbivore (insect and mammal) types -Variable: Insect_types		
9.1-Chewers		
9.2-Gall makers		
9.3-Leaf-miners		
9.4-Skeletonisers		
9.5-Leaf-rollers and tiers		
9.6-Sap feeders (honeydew, fumagine)		
9.7-Shoot deformation (shoots moth)		
9.8-Stem / bark borers (dust, resin, holes)		
9.9-Mammal grazer		

6 forest disease types - Variable: Disease_types	
6.1-Rust	
6.2-Mildew	
6.3-Leaf Necrosis	
6.4-Red Bands (needle cast)	
6.5-Canker	
6.6-Stem -shoot Necrosis	

3 Abiotic types - Variable: Abiotics
3.1-Drought
3.2-Frost
3.3-Wind
3.4-Mechanical damage
3.5-Fire

9. Forest herbivore (insect and mammal) types (Variable: Insect_types)

9.1. Chewers:

Insects that can eat most of the leaf material (succulent tissues, veins, midrib). They include the defoliators that destroy the entire leaf blade (only tougher mid-vein remains) and those that eat distinct portions of leaf such as distinct notches or circular holes cut from leaf margin, or small randomly scattered holes.



Figure 1 Leaf chewers: entire blade destroyed (left) and distinct portions eaten (right) Source: «TreeDivNet. 'Insect damage assessment – a field guide'»

9.2. Gall makers:

Insects that induce formation of galls and feed on their tissues.



Figure 2 Examples of galls Source: «TreeDivNet. 'Insect damage assessment – a field guide'»

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Figure 3 Example of closed galls on ponderosa pine caused by *Cecidomyiid midges* in Colorado Source: www.chemical-ecology.net/insects/gallmake.htm

9.3. Leaf-miners

Insects that feed upon succulent tissues while tunneling. In broadleaves, leaf miners feed between the upper and lower leaf surfaces: If the leaf is held up to the light, one can see either insect or frass within the damaged area (discolored or swollen leaf tissue area).



Figure 4 Examples of mined leaf Source: «TreeDivNet. 'Insect damage assessment – a field guide'»

9.4. Skeletonisers

Insects that devour all leaf tissues, except veins and midrib.



Figure 5. Examples of skeletonized leaves Source: «TreeDivNet. 'Insect damage assessment – a field guide'»

9.5. Leaf-rollers and tiers

Leaf-rollers: Insects that hide and feed inside one leaf or the tip of a leaf that it has rolled-up into a cigar-shaped tube.

Leaf-folders: Insects that fold up one leaf to make a shelter for hiding or feeding.

Leaf tiers: Insects that tie two or more leaves or needles together with silk threads, forming a tube in which hide and feed.



Figure 6. Examples of rolled leaf: folded leaf (left) and rolled leaf (middle); and leaf tiers (right) Source: «TreeDivNet. 'Insect damage assessment – a field guide'»

9.6. Sap feeders (honeydew, fumagine)

Insects that have specialized mouthparts for sucking the fluids from plants, thereby causing discolored spots or deformities or killing the affected plant sections.



Figure 7. Aphids (left) and damage of sap feeders on oak (right) Source: «TreeDivNet. 'Insect damage assessment – a field guide'»

9.7. Shoot deformation (shoots moth)

Insects that feed within buds and cause shoot deformation or decay.



Figure 8. Example of a larva eating a shoot Source: «TreeDivNet. 'Insect damage assessment – a field guide'»

9.8. Stem / bark borers (dust, resin, holes)

Stem borers: Insects that bore into the bark and feed on cambium tissue.

Bark borers: Insects that bore into and feed on the bark.



Figure 9. Example of bark boring Source: «TreeDivNet. 'Insect damage assessment – a field guide'»

9.9. Mammal grazer

Mammals that cause damage because of grazing.



Figure 10. Examples of grazing damages caused by big mammals (left and middle) and by rodents (right) Source: «TreeDivNet. 'Insect damage assessment – a field guide'»

6. Forest disease types (Variable: Disease_types)

6.1. Rust

Rust fungi on leaves and needles can be observed as yellow or rust-coloured uredospores in small clusters on the surfaces of leaves. These may be surrounded by necrotic lesions and chlorosis, i.e yellow, brown or black spots. Rust fungi may also be seen as aecia, i.e. tongue-shaped structures. From June-July onwards, resulting damage is visible but no more spores are visible, so it is sometimes difficult to differentiate from other causes of damage, as: small cankers, shoot deformation or shoot mortality.

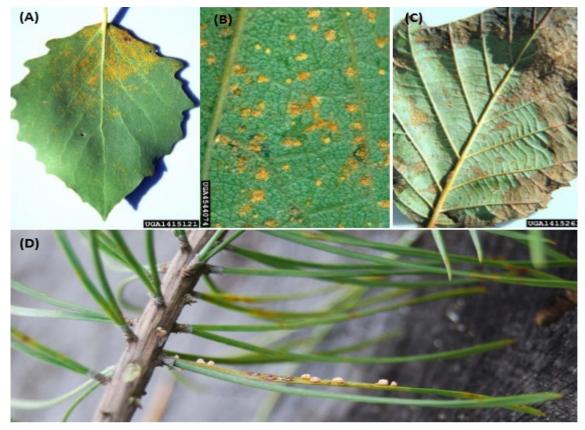


Figure 11. Rust. (A) Rust fungi on European aspen (*Populus tremula* L.) leaf (B) Close up of uredospores produced on poplar leaf, (C) Rust fungi infected gray alder (*Alnus incana* L. Moench) leaf and (D) Rust fungi forming aecia, i.e. tongue-shaped structures, on pine needles

Source: «FunDivEUROPE. 'Insect damage assessment - a field guide'»



Figure 12. Examples of typical symptoms of pine twisting rust in early spring (on developing shoots) Source: Private Marie-Laure Lousteau



Figure 13. Rust damages are sometimes difficult to differentiate from other causes of damage: small cankers, shoot deformation or shoot mortality
Source: Private Marie-Laure Lousteau

6.2. Mildew

Powdery mildew appears as a dusty or powder like white to grey spots or patches on leaf surfaces or other plant parts. In most cases this coating can be partially removed by rubbing the leaves, so that, it may be confused with dust/sand in some cases. Powdery mildew begins as discrete, usually circular, powdery white spots. As these spots expand they will merge, producing a continuous matt of mildew (similar to dirt or dust). In the patches minute pinhead formed fruiting bodies are developed, which are first white, then yellow-brown and finally black. These sexual fruiting bodies are only formed in late Summer/Autumn and they are hardly seen with the naked eye, unless they are very numerous There are several types of powdery mildew fungi, but the symptoms are basically the same.

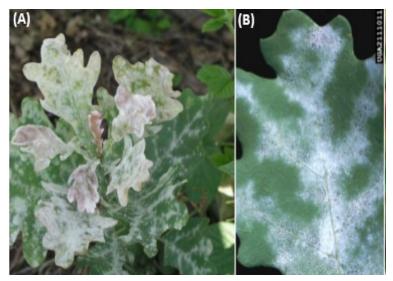


Figure 14. (A&B) Examples of powdery mildew on oak) Source: «FunDivEUROPE. 'Insect damage assessment – a field guide'»

6.3. Leaf necrosis

Leaf necrosis or leaf spots, pathogenic fungi on leaves typically form lesions, necrosis and fruiting bodies. On leaves symptoms typically occurs as yellow, brown and/or black spots and blotches. Minute fruiting bodies can often be observed within or in the vicinity of the symptoms.

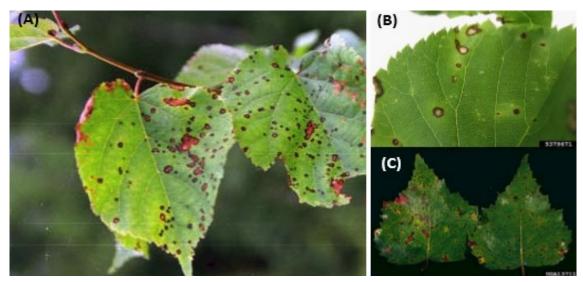


Figure 15. Necrotic leaf spots on leaves of (A) Little leaf linden (*Tilia cordata*), (B) Poplar (*Populus* sp.) and (C) European white birch (*Betula pendula*) Source: «FunDivEUROPE. 'Insect damage assessment – a field guide'»



Figure 16. Leaf spots on oaks (probably by *Septoria* sp.) Source: Private Marie-Laure

6.4. Red bands (needle cast)

Needle cast is a broad group of fungal diseases infecting needles and causing them to drop prematurely. The symptoms first appear on needles as light green to yellow spots, which then turn red, brown or darken. The needles can also have bands or be partially dead. Sometimes tiny black fruiting bodies can be observed on the surface of the needles.

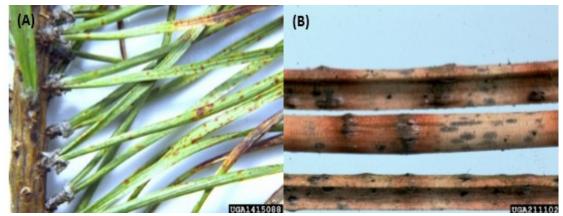


Figure 17. Examples of needle cast Source: «FunDivEUROPE. 'Insect damage assessment – a field guide'»



Figure 18. *Dothistroma* sp. on pine Source: Private Marie-Laure Lousteau

6.5. Canker

Cankers and lesions are localized on dead and damaged areas of bark but the symptoms vary depending on the pathogen and tree species. Cankers are often elongated with discolored tissue, sometimes with depressed areas and can be found on both stem and on branches. Some cankers also cause resin flow on outer bark and fruiting bodies can sometimes be observed. Canker usually suggests active plant reactions.



Figure 19. Examples of cankers: (A) Canker and fruiting bodies on European mountain-ash (*Sorbus aucuparia* L.), (B) canker on branch of European beech (*Fagus sylvatica* L.), (C) canker with resin flow on larch sapling and (D) necrotic lesions on the bark of the Douglas fir stem).

Source: «FunDivEUROPE. 'Insect damage assessment - a field guide'»

6.6. Stem - shoot necrosis

Necrosis is the death of a cell or tissue while still part of a living organism, therefore stem necrosis appears as a localized or delineated disease or lesion on the stem (see also Canker).

Shoot necrosis, shoot dieback or shoot blight appears as brown, stunted or dead shoots with wilted or dead foliage. The same symptoms can be caused by different agents.

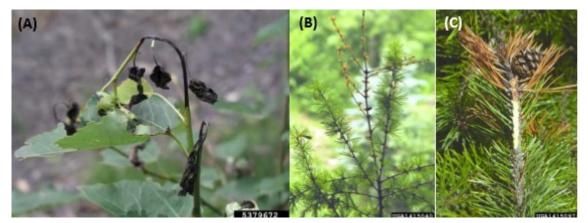


Figure 20. Shoot blight and dieback of (A) poplar (*Populus* spp.), (B) European larch (*Larix decidua*) and (C) mugo pine (*Pinus mugo*) Source: «FunDivEUROPE. 'Insect damage assessment – a field guide'»

3. Abiotic types (Variable: Abiotics)

3.1. Drought

The first effect of a lack of water is water stress. This produces a reduction in the cell growth and leads to a slowdown in the growth of twigs and fine roots. If the water shortage persists, the consequence is cavitation and embolism of the vessels, disrupting the operation of the entire system driver and causing wilting of the foliage. The leaves dry, curl up, hang and fall.





Figure 21. Examples of drought damage Source: Left: Private; Middle and right: www.fs.usda.gov/naspf/sites/default/files/iped-field-guide-print.pdf

3.2. Frost

Cold temperatures stop growth (below +4 to +7°C depending on the species) and photosynthesis (below -3 to -4°C). Frost damage occurs when ice forms inside the plant tissue and injures the plant cells. It may have a drastic effect upon the entire plant or affect only a small part of the plant tissue, depending on the sensitivity of the specie or provenance, growth stage and temperature.



Figure 22. Examples of frost damage Source: Left: http://ephytia.inra.fr; Right: www.fs.usda.gov/naspf/sites/default/files/iped-field-guide-print.pdf

3.3. Wind

Strong winds cause well identifiable mechanical damage: broken tops, blowdown (fallen trees, uprooted), broken, leaning trees, etc.

3.4. Mechanical damage

Mechanical damage is a generalized term to describe damage to vegetation from using equipment and from weather related events. Damage to vegetation from equipment can be simple carelessness or incorrect use of the equipment.



Figure 23. Examples of damage of lightning strike on bole Source: <u>www.fs.usda.gov/naspf/sites/default/files/iped-field-guide-print.pdf</u>



Figure 24: Examples of hail injury Source: <u>www.fs.usda.gov/naspf/sites/default/files/iped-field-guide-print.pdf</u>



Figure 25. Wounding of woody tissues (mechanical and human activity) Source: www.fs.usda.gov/naspf/sites/default/files/iped-field-guide-print.pdf

3.5. Fire

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