PROJECT FINAL REPORT

Grant Agreement number: 284181

Project acronym: TREES4FUTURE

Project title: Designing Trees for the Future

Funding Scheme: Combination of CP and CSA

Period covered: from 01 November 2011 to 30 April 2016

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1. Final publishable summary report

1.1 Executive Summary

European forests are facing major environmental and socio-economic pressures which might have considerable influence on forests species composition, on their structure and their outputs. As a result, wood products, both quantitatively and qualitatively are expected to change modifying the whole forestry wood chain.

In this context of uncertainties, the Trees4Future consortium, represented by 28 research institutes from 13 European countries, was settled down as a base to bring some answers to these burning challenges. To have one unique research infrastructure network covering the many forestry research issues is unrealistic and would be inefficient, so the ecological, biological, technological facets of trees and of European forests are many and complex. Instead, Trees4Future has focused its networking and research activities around forest tree breeding (and genetics) which has been used as a guideline all along the project. Forest tree breeding has indeed since several decades deeply influenced European forests through plantations and substantially improved its productivity. It will play a major role in adaptation of forests to environmental changes and industrial needs. A prominent characteristic of tree breeding is that it is by nature highly integrative as it relies on many scientific fields and imposes a multi-disciplinary approach.

The project – 'data' oriented- aimed firstly at improving the production, standardisation, archiving, accessibility and traceability of sound and reliable data for traits or parameters of various natures and useful for the forestry community but also for the scientific community at large. Secondly, it aimed at developing and/or improving and testing new analytical and modelling tools for a more efficient use of datasets.

Prominent results include:

i) Development and testing of innovative phenotyping methodologies for assessing complex traits such as phenological traits (flushing, senescence, cambial activity), hydraulic properties of trees (transpiration, conductivity, resistance to cavitation), and wood properties (extractive contents, natural durability, tension wood and collapse, mechanical properties). Near Infrared Spectrometry in particular proved to be a powerful and reliable tool for non-destructive, high-throughput phenotyping of several wood properties, including hydraulic ones. Together with the SilviScan technology, they are unique techniques to analyse from one sample many different wood properties.

ii) Standardisation of field trait assessment methodologies (and of molecular laboratories procedures) were agreed by partners for several traits related to adaptation and stem architecture. The many procedures used by research institutes for these traits (mostly subjectively assessed) have for long impeded exchanges of data among laboratories, complicated data analysis and prevented consolidation of results. In the same line, reference genotypes for several species have been identified and should improve assessment procedures, comparison of results and get access to pan-European results requested by some studies (plasticity, wood formation, epidemics).

iii) Creation of a unique web portal to access genetic and environmental data. Genetic as well as environmental datasets are highly spread among the many forest and environment research institutes and agencies. For researchers, it is a real challenge to get access to these resources. On the one hand, a specific portal for genetic resources, connected to the

main Trees4Future portal, was created and allows now access to the major genetic databases with an international dimension existing in Europe. On the other hand, a Clearinghouse for geo-referenced environmental data was created as a search and discovery tool that provides controlled and trusted datasets provided by the (European) forestry research community.

iv) breedR is a unique ensemble of different analytical modules designed for the statistical analysis of forest genetic trials. Thanks to innovative solutions taking better into account several phenomena that, being of high relevance in forestry, were rarely available within a single tool (e.g. competition), tree breeders will better estimate genetic values of trees. BreedR was conceived with generality and openness as main drivers to allow its constant development according to analytical progress and needs of users.

v) Several forest resources models designed to assess goods and services, sustainability, and adaptation and mitigation capacity of European forests were already existing before the project but their functionality, scope, performance and accessibility were greatly improved through in particular through integration of new factors (genetics, wood quality), better flows among models, and an open and more friendly structure. Several modelling tools were tested to model native and cultivation ranges of species combining environmental data and genetic data obtained from international field trials. These were tested as support to delineate breeding and deployment zones of species and provenances at the European level.

vi) Several experts groups around hot topics in a context of climate change (plasticity, phenology) were created to support thinking about research developments and experimental needs. Work around the place and expectations of breeding in Europe will be a precious support to further development of the infrastructure network. Key experts also collected information, tested methodologies and wrote roadmaps to design pan-European service labs (molecular, NIRS) and forest material traceability system in Europe.Finally a Strategic and Innovation Research Agenda was drawn thanks to the contribution of various kinds of stakeholders and broadly disseminated. The creation of a European Tree Breeding Centre is foreseen as the next challenge to further sustain the network and reinforce scientific and technical cooperation.

vii) The Trans-National Access programme, offering access to 28 modern and performing research infrastructures in several scientific domains (genetics, genomics and breeding, physiology and biotechnology, wood science and technology, modelling) was highly successful with more than 110 visitors. It fostered innovative research exchanges among labs and disciplines.

As a whole, Trees4fure was highly successful in developing links between disciplines and cooperation among laboratories, creating innovative tools and methodologies and settling down the basis for a European Tree Breeding Centre. Outcomes achieved –for some already available as scientific or technical papers with many more in preparation – are numerous and will benefit the forestry research community but also through many aspects industry and other forestry stakeholders.

1.2 Project context and objectives

In a context of rapid and sometimes unexpected environmental and socio-economic changes, forestry needs a more coherent, integrated and reactive research organisation to face major challenges encountered by European forests. Sustained wood production and procurement of other forest services are indeed threatened by climate changes and its cohort of emerging pests and diseases and made more complex in a global market asking for an increase of wood products, sometimes new, sometimes conflicting with traditional products or services.

European forestry research relies on academic fundamental research (e.g. in biology, environmental science, wood science) as well as on applied research (e.g. in breeding, silviculture, wood technology): both are needed together with the many research disciplines supporting them. Nearly all forestry research centres in Europe cover these many fields but their expertise, research capacity and facilities are uneven according to their national or regional priorities. Disciplines have developed their own research programme and methodologies: dialogue between them, even within a given institute is usually not straightforward. But all agree that multi- and inter-disciplinary approaches are more than ever requested to face new environmental and socio-economic challenges and bring innovative solutions.

Trees4Future clearly aims to bring these many different research communities together through 5 networking activities (WPs1 to 5, coordinated by G.J.Nabuurs, Alterra)) and 6 research activities (WPs6 to 11, coordinated by L.E.Pâques, INRA) to favour dialogues and exchanges and enhance cross-fertilisation through development or improvement of some key tools for the benefit of the whole scientific community. To cope with this multidisciplinary challenge and ensure coherence of the project, it was designed as a 'data-driven' project in the sense that 'data' (production, standardisation, collection, management, analysis and modelling) is at the core of the project as shown in the following diagramme.

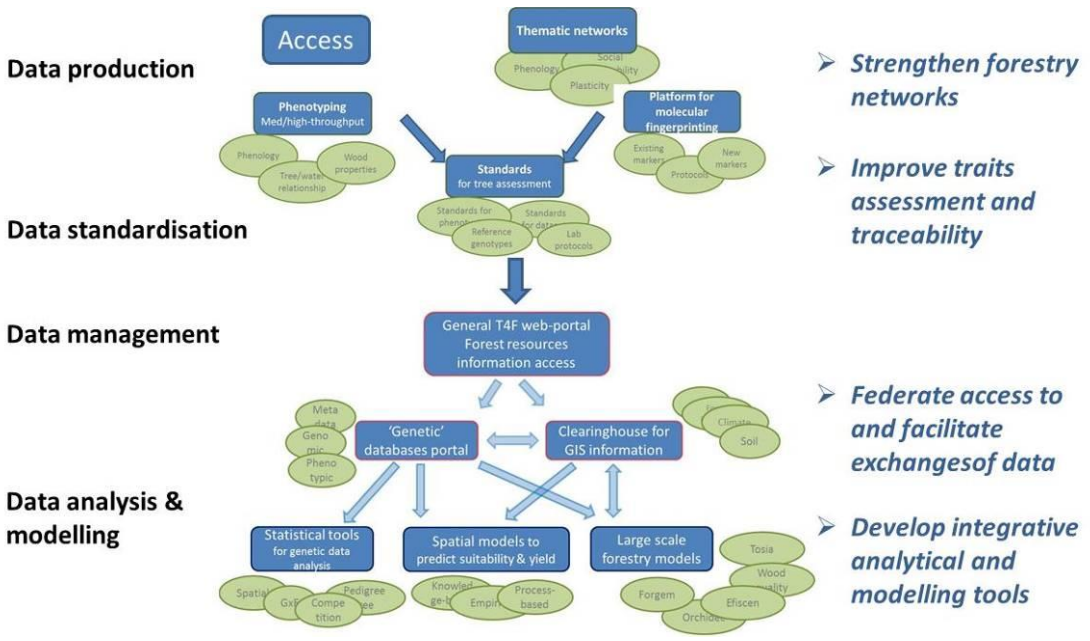

Figure 1. Trees4Future: a data-driven project

In short, WP1 together with WP9, supported by WP4 aimed at developing friendly and operational tools for properly archiving and facilitating access to genetic and environmental information. The objectives of WP6, WP8 and WP10 were respectively to develop tools to improve the statistical quality of experimental data analysis; to test adequate models for better explaining species distribution ranges and recommending provenances deployment zones for reforestation; and finally to improve the services offered by several major forestry resources models. WP2 aimed at standardising field assessment and some laboratories methodologies in order to facilitate exchanges of data while the objective of WP11 was to develop and test new phenotyping technologies for adaptive traits and for wood properties. Finally, WP3 aimed at reinforcing research networks around some key issues and WP4 had to lay ground to a Strategic and Innovation Research Agenda for forestry as well as to propose some ideas for sustainability of the research infrastructure.

The assembly –through the Transnational Access programme– of 28 modern, performing and complementary forestry research infrastructures across Europe is a strong sign of the willingness of Trees4Future to facilitate international scientific exchanges and research cooperation and a unique opportunity for researchers (and especially the youngest ones) to develop skills and initiate joined research projects.

Nevertheless, to remain realistic and to avoid an extreme dilution of efforts (human and financial), some choices were made to focus the project around forest genetics and breeding, an integrative field of forest research, relying on many disciplines ahead of it or following it in an interactive way. Trees4Future follows up in this sense a previous, more restrictive EU-initiative through the Treebreedex project aiming to the emergence of a European Forest Tree Breeding Centre.

1.3 Main scientific and technical results/foregrounds

1.3.1 A European platform for research data on trees (coordination: S.Fluch, AIT; R.Lokers, Alterra; T.Suominen, EFI)

1.3.1.1 Context and objectives

WP1, WP9 and WP4 have worked intensively together to produce the T4F web portal, which works as a central access point to the WP1 genetic database portal, the WP6 statistical package, the WP8 Climate matching tool, the WP9 clearing house and the modelling tools from WP10. A dedicated server has been purchased to run these T4F services, to ensure adequate computational performance and post project availability of the developed services. See Figure 2 on the configuration below.

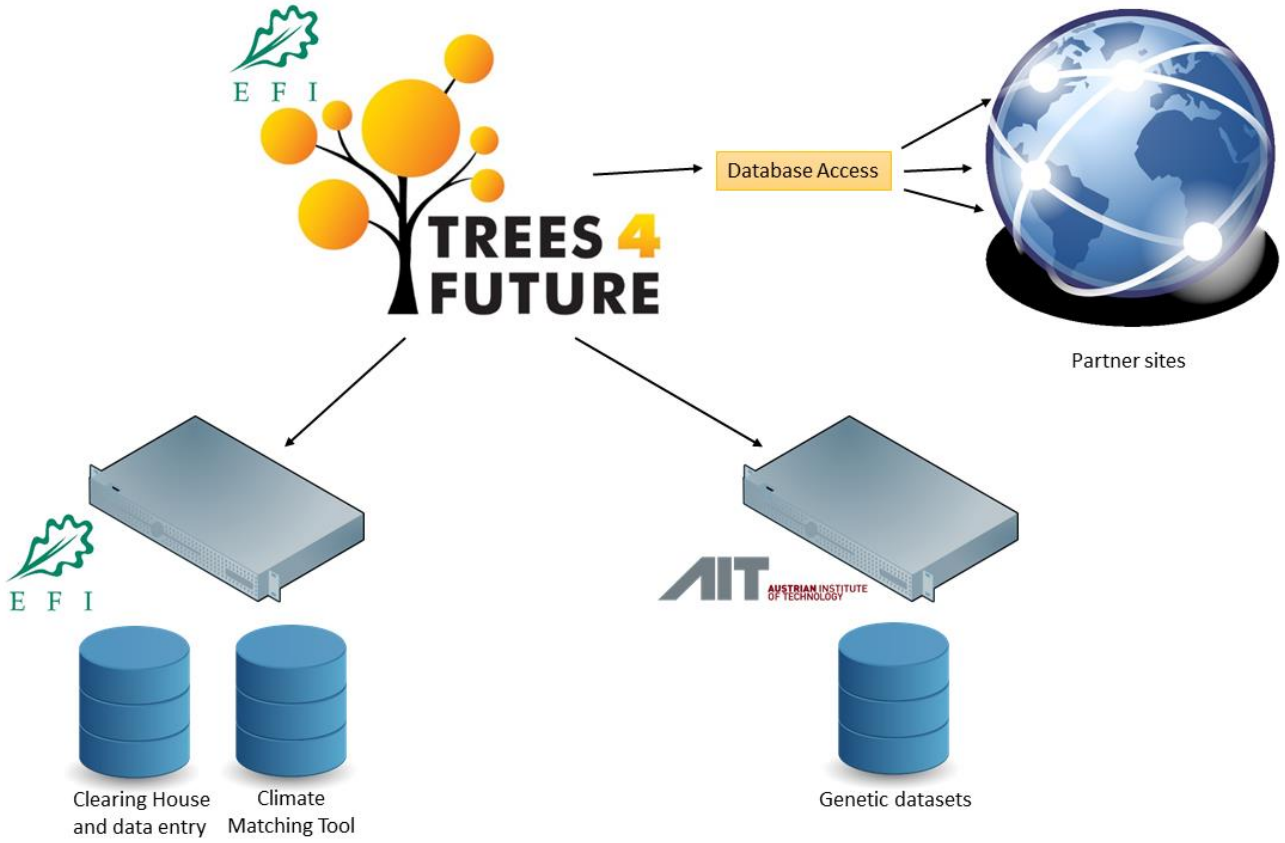

Figure 2. Configuration of the T4F web portal

The WP1 databases are available for searching through the T4F portal, but their metadata is also included in the clearing house. The WP1 genetic database portal has been embedded into the T4F web portal, and is actually running on AIT servers in Austria.

The WP9 Clearinghouse developed in the Trees4Future project is a central access point to discover forestry and forestry research related datasets. Users that require forestry data for use in their research or business (e.g. running models, performing analyses, impact assessments etc.) can utilize the Clearinghouse as a search & discovery mechanism that provides controlled and trusted datasets

provided by the (European) forestry research community, without having to contact a range of individual data providers and/or different portals and databases.

The rationale for developing such access points comes from the following:

- Research funded by public funding is more and more required to provide Open Access datasets, so mechanisms to manage and work with this wealth of data are indispensable for users to easily access the datasets that are considered relevant and trusted by the research community

- This means also that over time large amounts of research data will become available for research purposes and it will be impossible for any researcher to have the full overview of what is available.

- The generally accepted concept is that data remains with the data owner. The data owner documents, publishes and maintains the data. This leads to a network of distributed data repositories. The owners also publish information on their datasets through metadata catalogues. Thus, the datasets become discoverable and accessible.

1.3.1.2 Main scientific and technical results

WP1 - Structuring and providing a common access to databases

Starting with a comprehensive list of all databases which are available within the consortium, the data and availability of these specific sources has been analyzed. Overlaps and type of data have been identified and categorized. Some highly valuable databases have been selected to act as main sources of a federated database which is the main access point for the developed tools for supporting researchers and interested communities. One key point here is to define a data structure where several different types of information can be stored and combined in one place. Information about genetic markers is accessible as well as details for field trials or populations of trees. To enrich the linkage between the different datatypes an ontology has been designed where all elements are covered. The developed tools are mainly supporting the researcher by finding the data of interest.

Federated database

The selected databases are centralized at the federated database. Some of these databases are online available and they are still growing. These databases are automatically harvested via the TAPIR protocol. Normalization and validation is the first step which is done by PL/SQL scripts developed in the PostgreSQL database. In this step also the ontology will be attached to the data structure. The final result is a single database with all additional information starting from the data providers, database source, type of data, etc. All search interfaces are accessing the federated database directly.

Data transfer

The data transfer from the online databases in handled by the TAPIR - TDWG Access Protocol for Information Retrieval. On every site where a database is present “TapirLink”, a software package developed by the TDWG community is installed. A small harvester developed by the AIT tests every two weeks if there have been changes in the source databases. If something is new the database is transferred to the cache database.

Data harmonization

Because of the variability of the data within the databases harmonization is a need to make data comparable and categorize them. The harmonization process takes care of different languages as well as different notations for the same things. Often traits mean the same, they are just written in a different way. Double population names can be present and the data is merged from these datasets.

Most of this harmonization steps are done by mapping tables which have been developed during the project but these tables need maintenance whenever new traits, species, datatypes are implemented in the source databases.

Ontology

The ontology we created is an association of different already existing ontologies. The main parts are taken from the AGROVOC vocabulary. Missing parts have been added from ABCD, EDAM, SO and PO ontologies.

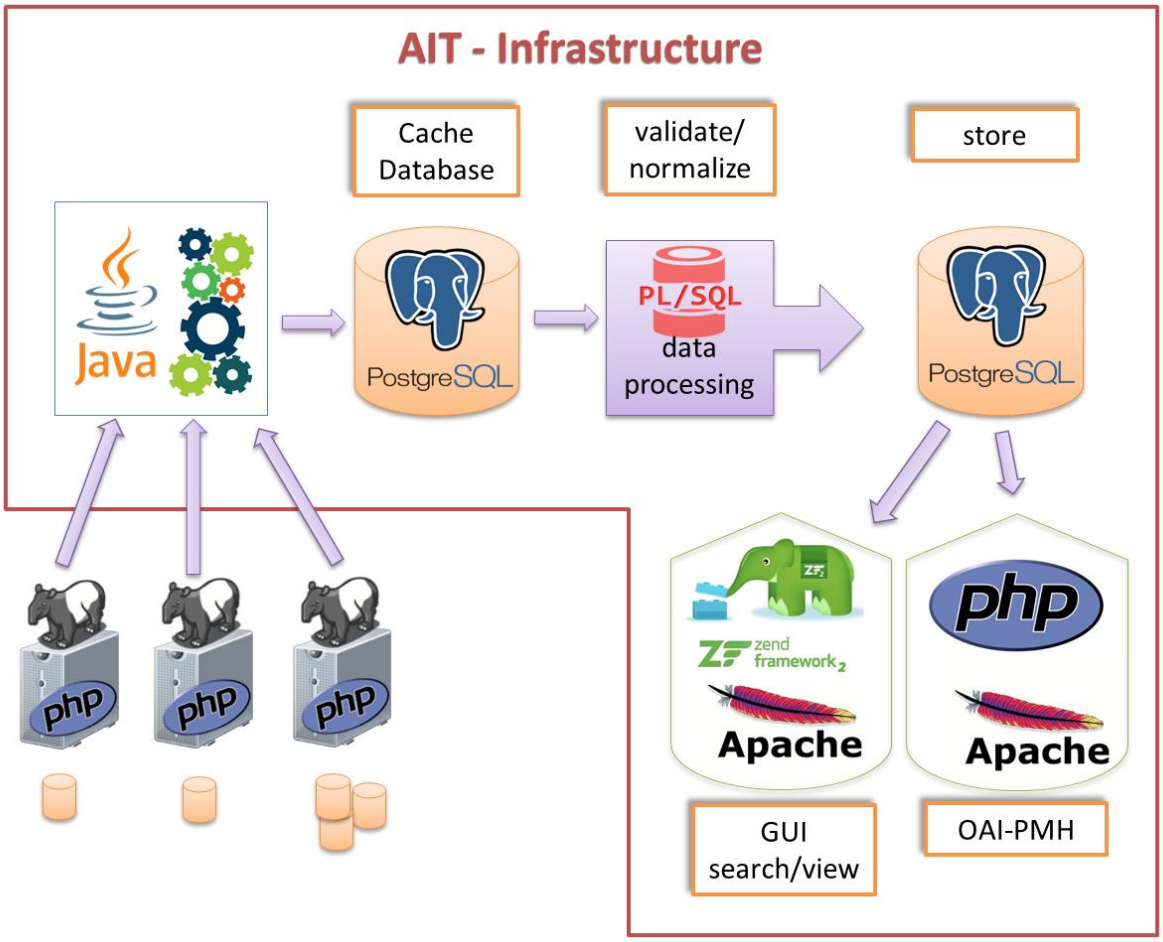

Figure 3. Data Processing from each data provider to the AIT services

Search interface

Once the data is stored on a central point we provide three different search interfaces for browsing the data. Every data item is tagged with the original source/contact.

http://www.trees4future.eu/database-access.html

Guided search

The guided search interface provides the easiest way to get a feeling about the data behind. The user has to start by selecting a Genus and Species and can refine his search results with the interactive

interface. The result is a table of data items. Each data item has as a function of its type more detailed information which is visible by selecting it. Relations to other data items are directly represented and linked to ease the browsing through the connected elements.

Full text search

If a population name is known or a specific tag is associated with an element the full text search can be used to search for all related data items. It consist of just one text field and expects strings of interest separated by space. The result is the same as in the guided search.

Ontology browser

The ontology browser can be used to get a quick overview of the ontology (as tree view).

Overview map

All datatypes with georeferenced data can be displayed in the overview map. On the right top of the map it is possible to change the map layer as well as the datatype which should be displayed.

Service provider

An OAI-PMH service acts as the interface between the Clearinghouse and the federated database. All metadata of the implemented databases is extracted and provided by this service. As data standard the Dublin core standard has been selected.

WP4 - T4F web portal

The development of the web UI for the Climate matching tool (WP8) and its online deployment is explained in section 1.3.5, a result of significant effort by WP4.

WP9 – Clearinghouse

Figure 4 shows the architecture of the Clearinghouse. Data providers managing data repositories describe their datasets and data services through metadata records that are stored in a metadata catalogue, ideally at the site of the data provider. Every metadata record describes a dataset or service, providing among others a URL pointing to the actual data set and/or service, through which the data itself can be accessed. Metadata catalogues are published by means of web-services that allow access to the metadata and support the collection (harvesting) of its metadata records. The Trees4Future Clearinghouse collects these metadata services, thus composing a centralized metadata repository.

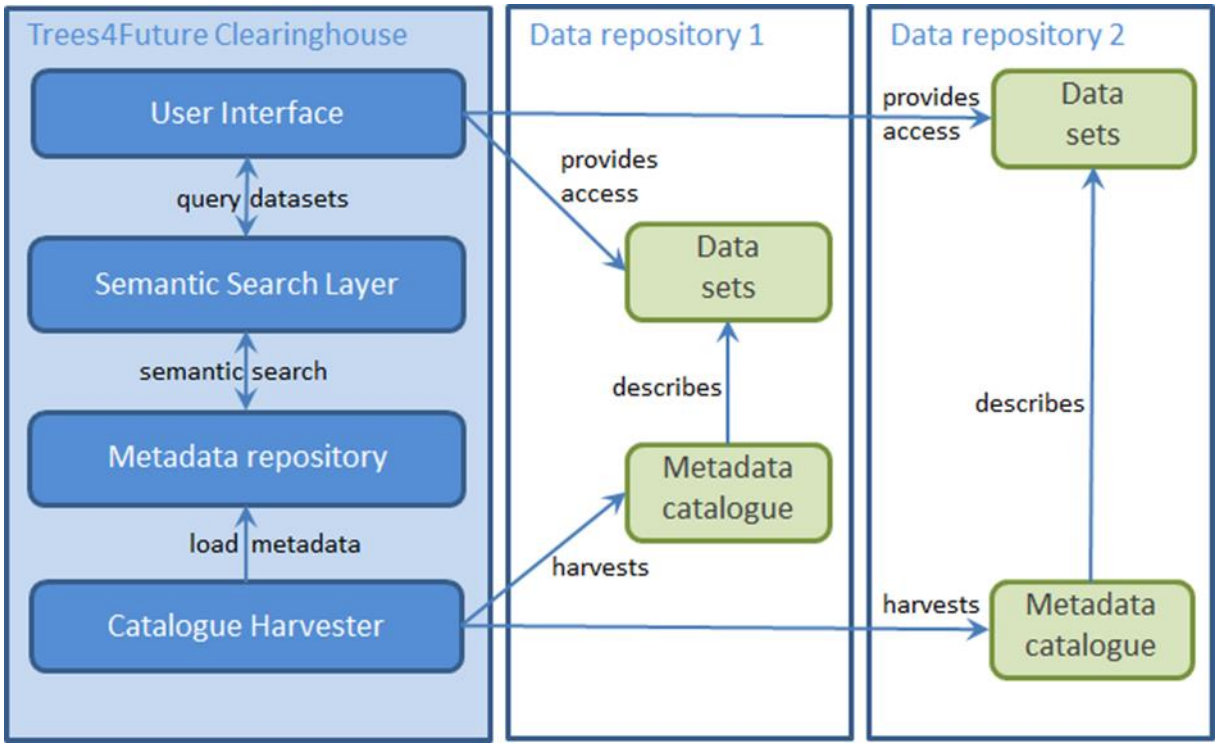

Figure 4. Trees4Future Clearinghouse and its links with metadata catalogues and data repositories

In order provide optimal search functions, concepts and technologies from the Semantic Web are used to store, index and search the metadata. The harvested metadata are triplified and stored as RDF in a triple store structured according to a model derived from the Trees4Future ontology. As part of this triplification process they are automatically coupled to domain ontologies, to facilitate that the end user can discover datasets using semantic search mechanisms. As an example, a user who searches for "rainfall" will, through the semantic relation between the terms "rainfall" and "rain" (in this case a synonym relation) also find the datasets that have the term "rain" as part of their metadata. Because of the available links to the actual datasets that are part of the metadata, users can through the search results eventually also access the dataset/services from the search results returned by the semantic search.

The Trees4Future Clearinghouse supports a range of commonly used metadata schemes and protocols and is thus able to harvest metadata from a wide variety of different metadata catalogues in the forestry domain. Consequently, the system is designed to be scalable and extensible. As a living data ecosystem, it can be extended to harvest and publish as many forestry-related metadata repositories as required and provide access to their underlying datasets.

From the user perspective, the Trees4Future Clearinghouse focusses on two major groups of users. The first group are the end users of the Clearinghouse: the researchers and other stakeholders from the forestry research community that require forestry research data to perform their daily work. These users will utilize the search and discovery functions (the web enabled search interface) of the Clearinghouse to query for datasets that are registered in the Clearinghouse. The second user group consists of the data providers and data owners that provide their data and publish the associated metadata, making their datasets and services discoverable and accessible for end users through registration in the Clearinghouse.

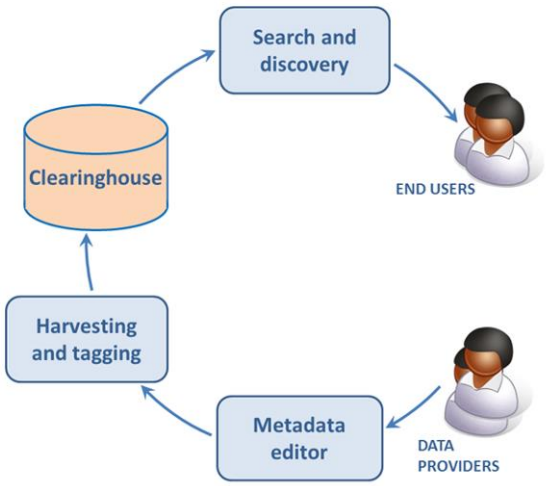

Figure 5. Trees4Future Clearinghouse functional components

Figure 5 displays a global schematic overview of the functional components of the Clearinghouse infrastructure. The arrows in the scheme demonstrate the flow of information on forestry data from data providers to end users through the functional components of the infrastructure. The individual components and their functionality are described in the following sections of this chapter.

The discovery of datasets builds on the availability of metadata and metadata catalogue services publishing the metadata of these datasets. Therefore, the first functional step in the process of serving the information on forestry data to the end users is the standardized registration of metadata on available datasets. Usually data providers use metadata editors, associated with the metadata catalogue they are using, to register and edit metadata. Metadata is stored in a catalogue and published using standardized catalogue services, so the metadata can be discovered, and if required be harvested and published by others.

Larger, more research and data oriented organisations are usually equipped with the technical facilities and capacity to set up and provide their own data and metadata infrastructure (external to the Trees4Future Clearinghouse infrastructure). However, quite some organisations in the forestry domain that possess relevant datasets do not have and/or cannot afford or technically support such facilities. To support these organisations, the Clearinghouse offers a dedicated function to register metadata. In this way, also organisations that do not have these facilities can contribute with metadata for their datasets and have it registered in the internal metadata catalogue of the Clearinghouse and accessible to end users. Including an internal metadata registration facility basically guarantees that every organisation and every researcher has the possibility to register and publish his metadata and consequently that the full wealth of data in forestry research can be made accessible to the European forestry community.

Figure 6 shows a screenshot of the Trees4Future Clearinghouse metadata registration form. It allows registration of the metadata of datasets according to the Trees4Future extended Dublin Core metadata format.

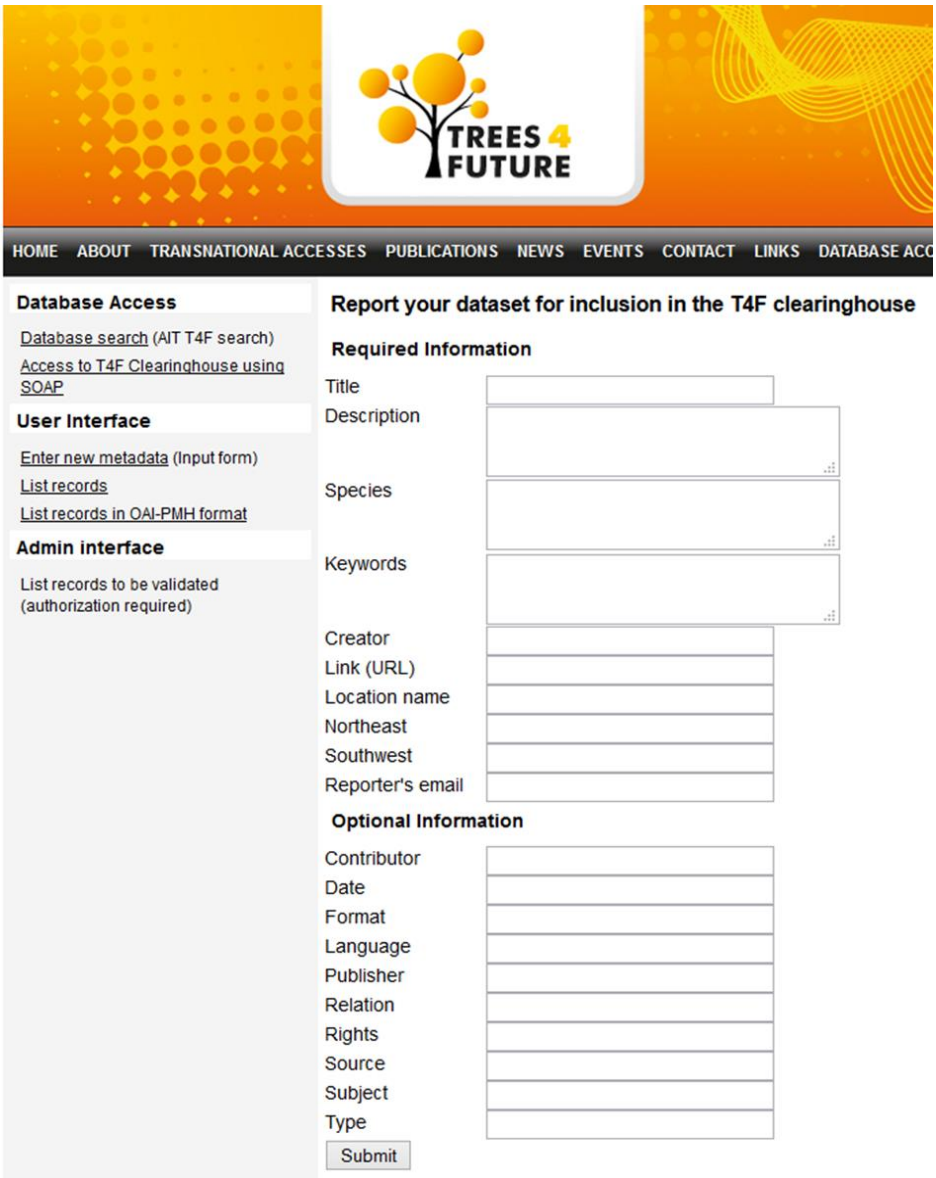

Figure 6. Trees4Future metadata entry form

In order to collect the metadata in the central repository of the Clearinghouse and make it searchable, a harvesting and metadata tagging process is in place. The metadata harvesting process uses standardized automated protocols to connect to the individual forestry metadata catalogues that are registered for harvesting and collects the metadata records that are available. This can either be from an external metadata repository or the internal Trees4Future metadata repository. The harvesting process creates a central database of metadata that will be frequently updated to reflect additions and changes made to the federated catalogues and keep the system up to date.

1.3.2 Standardisation of protocols for field assessment of traits: need for a common language (Coordination: B.de Cuyper, VLAGEW-INBO)

1.3.2.1 Context and objectives

Many traits are of interest to forest tree breeders to distinguish and evaluate genetic units (i.e. populations, provenances, families, clones), to assess genetic diversity and to determine the amount and adaptive value of phenotypic plasticity. These traits have proved their value as selection criteria as expressed by a high level of heritability and by their impact on the economic value of trees. Breeding programmes often rely on experiments with genetic units replicated over contrasting sites. These *common garden experiments* constitute a very powerful breeding tool but also urge for common assessment protocols and reference genotypes. It is indeed crucial that breeders throughout Europe "speak the same language". This will facilitate joint analysis of datasets, allow accurate comparison among sites and a more correct interpretation of results. In addition, it will 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 parameter trends within species.

Development of common protocols to be used in pan-European multi-site trials was set as a priority in the FP6 EU-project *TREEBREEDEX*. A major objective of the FP7 project *Trees4Future* (more particularly of Work Package 2) was to further finalise this work and, in addition, to select reference genotypes for each species/trait combination. Apart from the standardization of protocols, a likewise important objective concerned the reduction, as far as possible, of the subjective nature of the assessment schemes currently used, more particularly for the observation of phenological traits. This objective was dealt with and achieved within Work Package 11 of the *Trees4Future* project.

1.3.2.2 Main scientific and technical results

Common protocols

The starting point has been an inventory of existing and applied national assessment schemes. Firstly, this inventory built on one of the priority outcomes of the FP6 EU-project *TREEBREEDEX* (PÂQUES 2009, DUCCI *et al.* 2012) in which some common phenotyping protocols were already described. However, as this inventory did not include some species of high silvicultural value, such as beech and oak, it was complemented by a questionnaire as well as a literature review.

Common protocols were defined at a twofold level, i.e. species and trait level respectively.

Species level

In order to select the most appropriate protocols for common use, a set of criteria was defined to which they should comply: field-tested, non-destructive, objective, accurate, simple, low-cost, high throughput, reproducible, suitable for statistical analysis, suitable for large number of trees, relevant to one single trait only, defined for a specific age/size of trees.

Protocols were drawn up according to a fixed template, providing information on i. unequivocal definition of the trait, ii. general description of the assessment, iii. scoring scheme, iv. pros and cons of the selected protocol and v. references. In the *definition*, the general interest of the trait is briefly described, whereas the *description* of the assessment defines how and when and on what part of the tree the protocol has to be applied. Each scoring scheme consists of 3 to 5 scores, the highest score

indicating the best value, the lowest score standing for the worse value. Additionally, in the *pros and cons* part the ease of using the scoring system as well as difficulties that may appear are evaluated.

Finally, efforts have been made to disentangle complex traits where a global scoring might hide different realities (e.g. stem form components such as basal sweep, leaning, bending, crookedness, forking).

Common non-species-specific protocols (broadleaves / conifers)

Assessment protocols of certain traits appeared to be very similar regardless the tree species for which they were developed. Therefore, general protocols were adopted for the assessment of morphological traits for broadleaves and conifers (Table I). An example of non-specific protocols is given in Figure 7.

Table I. Compilation of common protocols for assessment of selected traits

Tree species	trait
Broadleaves	Stem straightness, forking, branching angle, branch thickness
Beech	Flushing
Oaks	Flushing
Ash	Flushing
Poplars	Flushing, bud set, *Melampsora larici-populina*, *Marssonina brunnea*, *Xanthomonas populi*
Wild Cherry	Flushing, leaf senescence, wood colour
Conifers	Stem straightness, basal sweep, forking, branching angle, branch thickness, number of branches
Norway spruce	Flushing
Douglas fir	Flushing, bud set, frost hardiness, needle cast
Scots pine	Needle cast, pine twisting rust
Mediterranean pines	Flowering, polycyclism, bark thickness
Larches	Flushing, canker, “drought” cracks

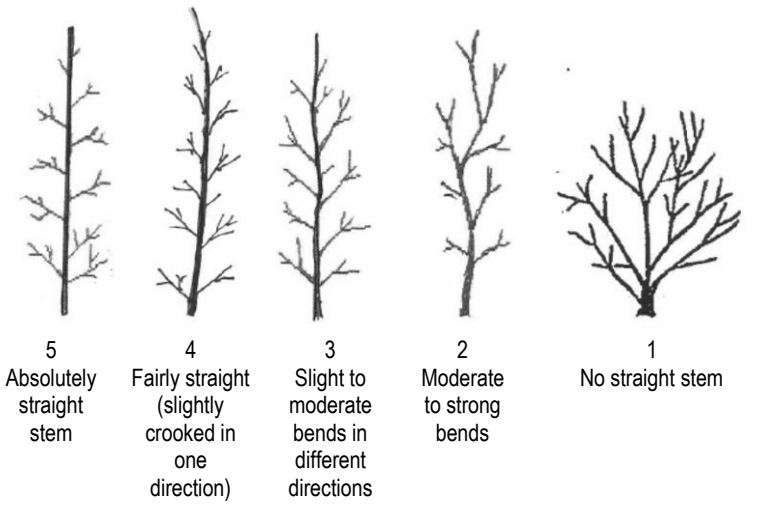

Figure 7. Example of a non-species-specific protocol: stem straightness of broadleaves

Species-specific protocols

Some adaptive traits are of major importance for broadleaves as well as for conifers. However, their assessment depends on the tree species and, therefore, cannot be reduced to one sole protocol, but urges for a species-specific approach. As many forest tree breeders were involved in this task, each with their own species of interest, a high number of species x traits combinations was obtained. Therefore, as not all species x traits combinations could be tackled, priority was given to:

- Six target species of high interest from a European point of view (beech, ash, wild cherry, Norway spruce, Douglas fir, Scots pine) and four species groups (oaks, poplars, Mediterranean pines, larches)

- The number of traits per (group of) tree species was limited. Traits were selected because of their high impact on tree survival, growth and on timber quality and, thus, on the adaptive and economic values of trees. A highly important set of traits concerns **phenology** (bud break and but set) as it is strongly related to adaptation. Phenological traits are conditioned by biological and environmental factors necessary for launching the processes (e.g. chilling requirements, forcing temperatures, seasonal changes), but they are also under strong genetic control. They are important selection criteria as they are directly related to tree architecture and growth: i. impact on stem form in case of stress (frost damage) and ii. effect on growth potential in relation to the length of vegetation period. Experimental data from earlier surveys show the adaptive meaning of bud break (flushing). Therefore this trait is a practically relevant indicator of the adaptability of forest trees to study the effect to global change. Protocols for assessing bud break were selected for all tree species (except for Scots pine). For poplar and Scots pine protocols for observation of bud set were added.

Disease resistance is an essential part of many tree breeding programs, as pathogens may have a substantial impact on tree growth (or even survival) and on wood quality. Long term breeding programs of several forest tree species have clearly indicated the genetic variation in resistance in genetic/provenance trials and in small seedling or clonal trials. This resulted in operational planting of resistant material.

Nowadays, forests and trees are increasingly affected by factors related to global change. Expanding international exchange (trade) of FRM has facilitated invasion of numerous insects and pathogens into new regions. Climate change is already affecting the geographic distribution of host trees and their associated insects and pathogens, with anticipated increase in pest impacts by both native and invasive pests. Only international collaboration will allow us to get up to date information on the spread and future importance of pathogens. Therefore common protocols were elaborated for the presently most important diseases, damaging forest trees in several European countries and with a potential to spread towards more northern neighbouring countries or to become more aggressive due to changing climatic conditions. The scoring schemes for each pathogen have been described exhaustively and are illustrated by drafts and/or pictures. Protocols deal with the most important diseases of poplar, wild cherry, common ash, larch, Douglas fir and Scots pine.

For some traits only important for a very limited number of tree species, **unique** assessment **protocols** were defined. Such examples are wood colour for wild cherry, the wood of which is used for “noble” applications (furniture, cabinet making, marquetry, turning); *Frost hardiness* is an important trait for nearly all tree species. However, in the case of *Douglas fir* it is not correlated with phenology and therefore it has to be observed independently; *Flowering* and *bark thickness* play an important role in the selection of Mediterranean pines. Flowering affects seed production and therefore is of major importance for tree species such as *Pinus pinea* whose economic value is constituted by its seeds (consumption). Bark thickness is a predominant trait in protective strategies

against forest fires. *Drought stress* on larch species sometimes causes cracks along the stem. Despite the fact that the origin is not yet known, this trait is of high importance as cracks usually turn around the stem over one to several meters and can be as deep as the pith.

Generic protocols

At the trait level, protocols were elaborated for assessment of wood characteristics and of drought resistance. Common protocols for determining wood properties were based on an inventory of available *non-destructive techniques* and equipment applicable to standing trees. More particularly, an acoustic method for the determination of mechanical properties (i.e. modulus of elasticity) was elaborated. A specific NIR technique was described in detail within WP11 of the Trees4Future project. For assessment of drought resistance, a common protocol emerged from the testing of a robotic measurement device (WP11).

A detailed description of standard protocols can be found on the Trees4Future website http://www.trees4future.eu/uploads/t4fdeliverables/T4F_D21_submitted.pdf

Reference genotypes

The concept of common reference genotypes, properly chosen for their characteristics, meets the need for a more precise assessment of genotypes in trans-national trials, established across highly contrasting environments, and, thus, for the comparison of results among tree breeders throughout Europe.

According to research needs, three types of reference genotypes are defined, directly linked to genetics and breeding activities or more broadly to silvicultural and ecological studies:

i. ‘Control’ genotypes. These genotypes allow breeders to evaluate their own genetic units (e.g. new varieties apt to certification) as they constitute controls towards which own genotypes can be ranked and genetic gains estimated.

ii. ‘Benchmark’ genotypes. Evaluation of certain phenological traits unavoidably relies on subjective scoring systems (e.g. branching habit). For these qualitative traits, signal genotypes act as references as they demonstrate extreme and intermediate values along scoring scales

iii. ‘Widespread’ common genotypes. For some ecological studies - in particular to assess the impact of climate changes (e.g. study of phenotypic plasticity, phenology modelling, monitoring of diseases and epidemics) - the creation of a dense grid of sites with the same genotypes across contrasting sites throughout Europe would be highly beneficial.

The nature, characteristics and use of reference genotypes were defined according to their objective. For example, vegetatively propagated individuals (clones) are requested for Benchmark genotypes to exactly represent the particular tree characteristics whereas for control genotypes, the best genetic unit (either a synthetic variety or clones or selected seed stand) representing the current state of genetic improvement is needed.

Apart from their intrinsic value, reference genotypes should comply with a number of criteria:

- stable in their genetic composition (e.g. seeds collected only in good crop years and mixture of crops over several years)
- low genotype-environment interaction, particularly important for references of type i. and ii.
- easily deployed/accessible, particularly important in case of vegetative propagation of single genotypes

Based on a survey addressed to tree breeders throughout Europe, a selection of representative reference genotypes was compiled, bearing in mind:

- That only a limited number of genotypes per species/trait combination can be retained
- The importance of a straightforward logistic organization of production, storage and distribution of plant material. In this context, priority was given to genotypes already mass-produced, certified and commercially available.

In total, 147 reference genotypes were proposed by several Trees4Future partners, committing themselves to provide reproductive material of these genotypes whenever needed

References

Pâques L.E. (comp.), 2009: European survey on assessment methods of morphological traits. Treebreedex Doc. 916: 53 pp.

Ducci F, De Cuyper B, Pâques L.E., Proietti R, Wolf H (comp.), 2012: Reference protocols for assessment of trait and reference genotypes to be used as standards in international research projects. CRA SEL, Arezzo, Italy: 82 pp.

1.3.3 High-throughput phenotyping: an urgent need for forestry research (Coordination: L.E.Pâques, INRA)

1.3.3.1 Context and objectives

The preliminary step of field and lab data acquisition is always key in forestry research, making meaningless all other data handling steps (statistical analysis, modelling) if insufficient in quantity and/or in quality. Datasets to work with must usually be large to embrace the diverse forestry realities linked to space and time. A particular field of research, namely genetic and breeding studies, interested by genetic variability within species and its interaction with environment (space and time), requests huge amount of data at different genetic levels (from population to individual) and for multiple traits. Typically breeding selection criteria include traits of various types linked to adaptation to abiotic and biotic factors, biomass production and architecture (i.e. stem form, branching). Wood properties are also becoming more and more frequently considered.

If certain traits can be rather easily and precisely assessed (e.g. tree diameter, height) and their methodologies of assessment standardised (see WP2), some others are clearly more complex when dealing with forest trees. Complexity arises from all types of constraints which limit phenotyping capacity and reliability: destructiveness of the method (tree must be cut down), time-consuming and heavy processing (for example requiring many steps in sample preparation), costly methodology, subjective evaluation, complex protocols to implement.

Obviously development of high-throughput phenotyping (HTP) methodologies (and/or of tools allowing HTP) is a priority. Whereas genotyping development has been booming over the last decades and has revolutionised genetic and genomic studies, innovation in phenotyping has been lagging well behind as expressed by Cobb et al. 2013 who recognise the urgent need to '*Move from a data-starved, largely observational discipline to a data-rich science capable of prediction'.* This is particularly true in forestry research because of tree size and longevity.

Priorities in our research

Following a preliminary survey on phenotyping methodologies (reported in D11.1), our work focused on three categories of traits chosen for their phenotyping complexity but also for their high relevance in a context of global changes where trees better adapted to climate and wood products better suited to societal needs are searched for. We considered 3 groups of traits, namely phenology, tree hydraulics and wood properties, as 3 priorities.

Phenology relates to the timing of various developmental aspects (flushing, senescence, reproduction, cambial activity) of trees: they are conditioned by both genetics and environmental factors (temperature, photoperiod). Phenology is a major adaptive trait which is at the basis of natural distribution of species and of their cultivation zones.

Tree hydraulics links to various functional aspects of water in trees. It relates to key ecophysiological parameters describing tree efficiency in water use, tree tolerance/resistance to drought stresses. It conditions tree survival and growth and it is particularly critical in a context of climate changes.

Finally, wood properties relate to the many anatomical, physical, mechanical, chemical and biological properties of wood which feature wood of each species. Our focus was put mostly on chemical and biological traits together with some mechanical properties and wood defects. Our choice was driven by the complexity of their assessment and their economic importance for the wood industry.

Development of high-throughput phenotyping methods for these traits followed different objectives, namely:

- For phenology: to reduce the subjective character of classical assessment methodologies (scoring systems) through automated detection of phenological events and to adapt assessment to tall trees (currently limited to a few meters tall trees as in breeding research).
- For tree hydraulics traits: on one side to test a robotic system to automatically and precisely monitor artificial drought stress and generate high-frequency data on transpiration; on the other side, to develop proxies for assessment of tree vulnerability to drought.
- For wood properties: to develop non-destructive methods and proxies for assessment of traits either complex and/or costly to assess. Methodologies allowing multiple traits assessments at once should be privileged.

1.3.3.2 Main scientific and technical results

Towards less subjective evaluation of phenology

Classically vegetative phenology (flushing, foliar senescence) is visually assessed by various scoring systems following a gradual scale representing major developmental stages of buds and of leaves or needles. In many cases for conifers, only the terminal bud is considered. Besides being non-standardised (one of the objective of WP2), such scoring systems impose assessment at the right date, that is when the greatest variability among trees is expressed, are limited to observation of

rather small trees (a few meters high) and remain highly subjective. Alternatives methods have been tested with at least one of these aims: to reduce subjectivity, to allow assessment of tall trees, to avoid tedious planning of assessment time, and to obtain more precise kinetics of phenology.

Several new approaches have been tested (see Figure 8 below) and evaluated during 3-4 years (2013-2015 (2016); they can be grouped into 4 categories:

a) Less subjective and more global visual scoring systems: global assessment of the whole crown (greening/senescence scale; adapted BBCH scale); partitioning of the crown (global notation by compartment; phenology assessment of individual branches);

b) *Ex situ* assessment of flushing: twigs collected in usually far-away field trials, following endodormancy break, are forced into climatic chambers where phenology is closely monitored; this approach has been used also in dedicated experiments to determine some key phenological parameters such as chilling and forcing needs and temperature thresholds;

c) Automated monitoring of phenology: time-lapse cameras capturing hourly pictures, further analysed with image-analysis software (*eg.* Phenocam) and, LED captors allowing a continuous measure of incident/transmitted light under tree canopies were tested.

d) Automated captors of trunk radial size variation (dendrometers) together with microcores sampling were developed and tested to monitor cambial phenology and relate it to vegetative phenology.

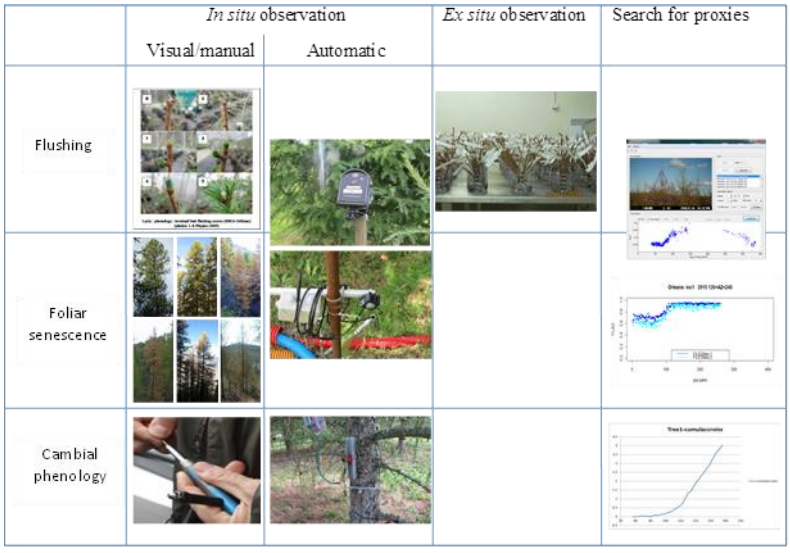

Figure 8. Some of the methodologies tested for assessing phenology

Research was conducted on common species (one broadleaf: wild cherry; one conifer: larch) and whenever possible on common pedigrees (clones for wild cherry; ½ sib progenies for larch) to limit noises which could be generated by different genetic background but also to learn on site effects.

Several of these methods or equipment have been described in 1-2 pages leaflets for dissemination. Detailed analytical results will be published in scientific papers. A global evaluation of benefits and constraints of the different methodologies tested is given in Table II. From these experimentations, we raised questions and learned several lessons:

1) Definition of flushing/senescence: among the different continuous developmental steps characterising flushing, bud setting and foliar senescence, what is (are) the critical step(s) to be considered; in other words, when should we consider a tree as flushed? All methodologies tested allow detecting early vs late flushing (senescent) trees but these categories might differ according to the method and organ which is monitored.

2) Phenology, particularly for flushing, may pass from one stage to another at such a speed (24-48h) that only automatic devices allowed us to detect. Subjective methodologies are unable to monitor closely phenology kinetics.

3) Foliar senescence is overall more complex to assess than flushing: interference with biotic events (eg. early attacks by *Cylindrosporosis* in wild cherry: *Meria laricis* in larch) and abiotic factors (summer drought, autumn frost) can disturb senescence observation (early fall down of leaves/needles; abnormal coloration of leaves).

4) Subjective scoring methods remain attractive when large numbers of trees (over 100) should be monitored. Partitioning of tree crown allows capturing acropetal (/basipetal) behaviour of flushing/foliar senescence in some species as wild cherry and larch. BBCH scoring system, adapted to forest species, permits a global evaluation of bud phenology at the crown level, which might be more pertinent than just looking at terminal buds. Its derived application (50% of buds at each of some critical stages) looks more handy than in its classical use (10%, 20%...90% at a given stage).

5) *Ex situ* methodologies have several advantages in terms of planning of observations, frequency of flushing monitoring and capture of some key phenological parameters (usually arbitrarily fixed in phenology modelling); nevertheless not all species are adapted to be monitored as explants and the quality of twigs is primordial.

6) Automatic devices are well-adapted for studies on a few tens of trees (up to 100?) when high-frequency observations are needed. As mentioned in 2), we could not suspect such a high speed in flushing development in some years. The commercial cost of such devices (150 euros (cameras) to over 350 euros (dendrometers) prevent their use on thousands of trees as requested in some studies (genetics, breeding). Alternatives such as the cheap LED captors and home-made dendrometers, developed during the project, offer new phenotyping possibilities (up to 100-200 trees). In any case, automatic devices still impose a close monitoring of the tools themselves (batteries, lenses and captors obstruction, radial course of dendrometers, etc).

7) There are usually delays in the timing of phenological events kinetics when drawn from repeated subjective scoring and automatic devices (GCC (green chromatic component as measured with Phenocam software from pictures); fiLED (ratio of incident/transmitted light as measured from LED captors)).

8) Radial growth phenology as recorded by automatic dendrometers is usually not synchronous with vegetative phenology. It provides important features for better understanding wood formation in relation to climate. Progress in the speed of processing microcores collected in parallel is key to better monitor xylogenesis.

Table II. Benefits and constraints of methods tested for assessing phenology

Methods	Organ of trees monitored	Observation period 1)	Applicable on	Subjectivity level	Throughput Nber of trees		Throughput Observation frequency		Planning: seasonal constraint	Major advantage	Major constraint
Scoring scale	Terminal bud	**spring**/ autumn	Young trees (a few m)	+++	+++	Several hundreds	+	1 to a few dates	Strong	Fast, cheap	Subjective/ planning
Crown sampling or partitioning: Scoring scale or counting of flushed buds	Crown	**spring**/ autumn	Young trees (a few m)	+++ O	++ +	Several hundreds to a few tens	+	1 to a few dates	Strong	Capture Intra-crown variability	Subjective/ planning
Greening scale	Crown	**spring**/ autumn	Tall trees	+++	+++	Several hundreds	+	1 to a few dates	Strong	Fast, cheap	Subjective/ planning
BBCH scale	Crown	**spring/ autumn**	Tall trees	++	++	Several hundreds	+	1 to a few dates	Strong	Fast, cheap	Subjective/ planning
Photos (Manual)	crown	**spring/autumn**	All types of trees	O	++	Several tens	+	1 to a few dates	Strong	Keep record/objective	Timing, visibility, climate
Time-lapse camera (Automatic)	Crown	**whole year**	All types of trees	O	+	A few tens	+++	Every day (even hour)	Weak	High frequency/ objective	Cost/visibility/ climate
LED captor	Crown	**whole year**	All types of trees	O	+	A few tens	+++	Every day	Weak	High frequency/ objective	(cost)/ installation

								(even hour)			
Radial trunk variation Dendrometers	Trunk	**whole year**	All types of trees	O	++	A few tens → a few hundreds	+++	Every day (even hour)	Weak	High frequency/ objective/access to other traits	Cost/ installation
Microcores	Trunk	**whole year**	Old trees	O	+	A few tens	++	15-25 dates /year	Weak	Medium frequency/ objective/access to other traits	Need specific equipment for preparation of samples
Ex situ (climatic chamber) phenology monitoring (counting of buds)	Twigs	**spring**	All types of trees	O	++	100-200	++	Every 2-3 days during 1 month	Some flexibility	Controlled conditions, more precision	Work on explant and not on entire tree *in situ*
Flushing parameters (climatic chamber) (counting of buds)	Twigs	**spring**	All types of trees	O	++	10 to 100	++	Every 2-3 days during 1 month	Some flexibility	Controlled conditions, more precision	Work on explant and not on entire tree *in situ*

Phenotyping tree hydraulics to better adapt trees to drought

During the past years, forest dieback due to drought and heat waves has been frequently observed. For the future it is predicted that, in relation with climate change, drought and heat waves will become more extreme and will occur more frequently. For many temperate species drought is one of the main climate risks determining survival. Therefore, traits like hydraulic conductivity and vulnerability have become increasingly important in tree improvement programmes.

Experimentation of different water regimes to simulate drought events has become more popular among forestry researchers but proved highly complex when a close monitoring of drought levels is needed. Testing the suitability of the robot Pollux (INRA-Nancy) to monitor drought and tree responses to drought was one of the two objectives for this group of traits. The second objective aimed at developing proxies for assessing hydraulics properties, complex to assess with traditional methods such as conductivity and resistance to cavitation and thereby not suited for high-throughput phenotyping.

a) Experimentation with the robot Pollux

A set of two dedicated compartments of a climatically controlled greenhouse was equipped in 2013 with robotic weighing and watering devices which allow on one side to precisely monitor drought level in 10 l potted trees (every 90') and on the other side to estimate water loss and thus transpiration at a high frequency. Capacity of one compartment is theoretically of 72 containers.

Two case-studies have been conducted, the first one in 2013 with 4 poplar clones contrasted for their drought resistance and three water regimes (two levels of drought (11 and 8% of soil volumetric humidity+ control); the second one in 2015 with seedlings from 3 larch species and two water regimes (one drought + control). Focus was on biomass increments formed under the drought period.

Figure 9. Drought experimentation in robot at INRA-Nancy (left: 2013 poplar experiment with 4 clones and 3 water regimes: 60 plants in total; right: 2015 larch experiment with 3 species and 2 water regimes: 36 plantlets in total)

The following figure shows for 2 clones from the Poplar experiment hourly transpiration changes over the experimental period for the 3 water regimes (back: control/ orange: moderate drought/red: more intense drought). It clearly indicates that the different control and drought levels were properly maintained throughout the experimental period.

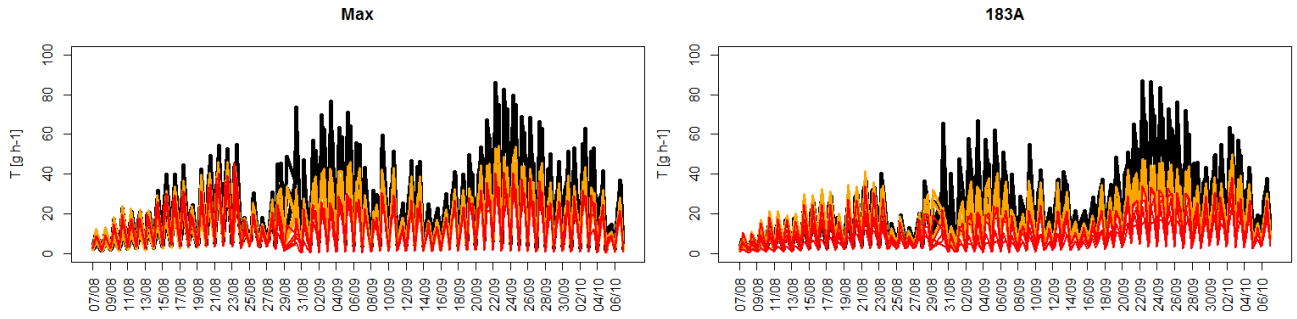

Figure 10. Transpiration (g/h) along the growing season for 2 poplar clones (Max and 183A) submitted to 3 water regimes (black: control; orange: moderate drought; red: more severe drought).

Such precisely monitored drought experimentation proved useful in studying the variable behaviour of species or clones for a given water regime, the impact of a few contrasted drought levels on wood formation of plants and their interaction. For example, Figure 10 above illustrates the different behaviours of clones against drought with an early differential transpiration among treatments for clone Max compared to a much later reaction for clone 183A. The following figure illustrates 2 key ecophysiological parameters for the 3 larch species tested (EL, JL, HL= European, Japanese and their hybrid) and their reaction to a 40 day-drought episod (D).

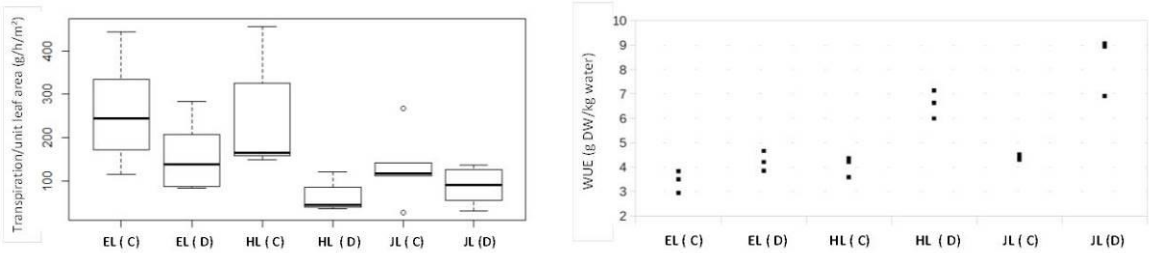

Figure 11. 2015 larch drought experiment: left: transpiration/unit leaf area; right: Water Use Efficiency (WUE) for European (EL), Japanese (JL) and their hybrid (HL) submitted to 2 water regimes (C= control; D= drought)

Measures of water loss and thereby of transpiration in the different genetic and environmental backgrounds can be completed by assessment on the plants of various biomass parameters (e.g. diameter, height, leaf area, aerial dry biomass, root biomass) as well as on wood samples, of hydraulic properties (e.g. conductivity, resistance to cavitation) and of wood anatomical and chemical features. Relationships among these many different parameters allow a better understanding of genotypes reaction to drought and of drought effects on wood formation. For example, Transpiration efficiency was strongly related to vessel and vessel lumen area (-), number of fibers (+) and mean fiber area (-) but also to P50 (-) and P12 (-).

They allow also identifying promising proxies of traits more complex to assess such as water use efficiency and vulnerability to cavitation. For example, the close relationship (>0.7) between mean vessel area and P50 (water potential at 50% loss conductivity) identifies mean vessel area as a good proxy candidate.

b) Development of proxies for hydraulic properties

Characterisation of species and trees hydraulic conductivity and in particular its disruption due to embolism in case of drought allow better understanding trees efficiency in terms of water use (WUE) and their vulnerability to cavitation. They condition trees survival and their capacity of biomass production. The most widely used hydraulic vulnerability parameter is P_{50}, defined as the water potential that causes 50 % loss in hydraulic conductivity. Another important parameter is the relative moisture loss (RWL) related to water potential since e.g. RWL_{50}, i.e. the water potential causing 50% moisture loss, contains information on the hydraulic capacitance (the capability to store water) of sapwood. Several techniques to measure xylem hydraulics are available, but there is still a need to develop more rapid, less labor-intensive and operative methods to measure vulnerability to cavitation.

Two proxies have been successfully developed:

FT-NIR spectrometry models were developed to predict P_{50} (applied air pressure causing 50% loss of hydraulic conductivity) and RWL_{50} (applied air pressure causing 50% relative water loss), based on solid wood samples of Norway spruce clones. Partial least squares regression (PLS-R) models with cross validation were used to establish relationships between the FT-NIR spectra and the reference data from hydraulic properties analysis. Based on the values of the coefficient of determination (r^2) and the root mean square error of cross validation, predicted results were evaluated as acceptable. In particular, the models developed from spectra obtained from the axial surface gave better results than the models from the radial surface for P_{50} (r^2 = 0.65), as well as for RWL_{50} (r^2 = 0.77). The method proves to have a high potential to determine routinely P_{50} and RWL_{50} as it is rapid, reliable, and non-destructive.

The other approach tested was to find anatomical proxies assessed by SilviScan technology. The SilviScan instrument provides data on radial variations in the cross-sectional dimensions of the tracheids (tracheid diameter and cell wall thickness) and in density with a radial resolution of 25 µm. The line of thought was that the cross-sectional dimensions of the lumens of the tracheids define a maximal potential of the wood to conduct water. Using a formula derived from CSIRO, conductivity at ring and within ring levels could be estimated as:

Lumen hydraulic conductivity $K = \frac{r^3 t^3}{8\eta R T (r+t)^2}$ where

R	radial diameter
r	radial lumen diameter
T	tangential diameter
t	tangential lumen diameter
ή	dynamic viscosity of water

At the clonal level on the same clones of Norway spruce as above, an excellent correlation was found between conductivity of sapwood at saturation (Ks) and conductivity estimated from anatomy (r =0.99). As well, resistance to cavitation (P_{50}) was strongly correlated to wood density (WD) (r = -0.77, P < 0.01), but also to conduit wall reinforcement ($(t/b)^2$ (i.e. square of wall (t) to lumen (b) ratio) (r = -0.68 with tangential hydraulic tracheid diameters). A similar approach was attempted on poplar clones, a broadleaf species. Mean vessel area of the whole wood sample was positively related to resistance to cavitation P_{50} (r = 0.54) and to the maximum hydraulic conductivity (r = 0.45). The strongest correlations were found between mean vessel area of the inner part and P_{50} (r = 0.73). In conclusion, anatomical features seemed to be reliable proxies to estimate several hydraulic properties in conifers but also in broadleaves and SilviScan instrument allows high-throughput.

Several scientific papers have been published or are in preparation to disseminate these important findings.

Can we speed up the characterisation of complex wood properties?

Wood is a complex material, the anatomical, chemical, physical and mechanical properties of which characterise the different species and determine their current and potential uses. These properties are usually assessed in wood research laboratories with dedicated –usually costly- equipment. Access to these laboratories to non-wood scientists is limited and they impose to work on wood samples which actually impose felling of trees and long-process preparation of wood specimen to meet standard requirements. Clearly they are not ready for routine assessment of wood properties such as requested by breeders, geneticists and industry for whom high-throughput and low-cost phenotyping is a priority; in addition for breeders and geneticists non-destruction of trees is a necessity and another constraint.

Alternative phenotyping methodologies are thus highly requested: non-destructive, low-cost, fast, reliable and implementable on non-destructive wood samples such as increment cores or right away on standing trees. Due to large sets of wood specimen to be assessed -as typically in genetic/breeding studies (several thousands)-, cost of core collection is already prohibitive. Thus any analytical methodologies which could determine from the same sample several properties (i.e. same property along the radius; properties of different natures) should be privileged in the way some already existing tools (microdensitometry, SilviScan) are functioning.

Among the most promising technologies, Near InfraRed spectrometry (NIR) was investigated to determine its potential to assess several among the most complex wood traits. NIR spectrometry has found many applications in agronomy and agro-industry but it is also rapidly developing in forestry and wood products research. Our focus was on:

i) chemical traits such as *extractives* which are usually in close link with wood decay resistance but costly to determine through wet chemistry;

ii) on some *physical and mechanical properties* (i.e. MOE, shrinkage) for which the standard assessment methods impose to work on clearwood specimen obtained destructively;

iii) on major but complex wood defects such as *collapse;*

iv) on *wood decay resistance* for which decay test following standards requires a costly preparation of samples obtained destructively, long delays (16 weeks), and are highly subjected to within tree variability and fungi biological activity.

Successful NIR spectrometry calibration models have been obtained for phenolic extractives in larch and for stilbenes in Scots pine. Both groups of components are involved in natural durability of these species. Models were built on large sample sets (500 + 100 for larch; nearly 500 for Scots pine). Spectra were acquired on powder but more interestingly on solid wood samples (half cores or thin sawn boards from increment cores) to further reduce sample preparation delay and cost and improve high-throughput. With a similar concern, the minimum number of spectra/sample was tested. For Stilbenes components, R_v^2 values ranged between 0.79 and 0.91. For larch phenolic extractives, they ranged from 0.67 to 0.92 (excluding DHK molecule). Collecting only two spectra in the calibration set could provide sufficient information for model development.

Among other wood chemicals for which NIR spectrometry proved successful is lignin content. A robin-test across 5 laboratories differently equipped with NIR spectrometer devices and expertise in

pre-treatment of spectra showed high prediction possibilities of lignin content (R^2 ranging from 0.86-0.92).

Prediction of Modulus of elasticity (MOE) from NIR spectrometry on solid wood specimen was also attempted. Material tested came from over 630 samples of larch from 13 origins collected across Europe. Reference MOE values were determined by an acoustic method on clearwood specimen. Best models showed R_v^2 = 0.75 and RMSE of 1470 MPa, showing again the possibility of reliable estimation of MOE by NIR spectrometry.

Tension wood is a major cause of sawn timber degradation in *Eucalyptus globulus*, contributing to non-recoverable collapse and stability problems during sawing and timber drying as well as quality (density) variations in the final products. Peripheral growth stresses (an indicator of tension wood) in standing trees may be estimated from growth strain release, which can be measured with the growth strain gauge method developed by CIRAD-Forêt. However the method is time-consuming and only may give an indication of the level of growth stresses in the periphery of the tree. NIR predictions of cellulose content and stiffness (modulus of elasticity, MOE) from spectra collected from the radial longitudinal surface of *Eucalyptus globulus* wood were found to be reliable indicators of zones of non-recoverable collapse associated with the presence of tension wood. Collapse bands occurred where NIR-predicted cellulose content and MOE exceeded threshold levels of 50 % and 25 GPa, respectively for more than four consecutive millimeters. On another samples, a combination of two properties proved to be a good predictor of the boards presenting areas with clear non recoverable collapse (tension wood): density $\geq$ 1000 kg/m^3 and Klason lignin content $\leq$ 14%.

Finally, NIR spectrometry was used to test its suitability for prediction of decay resistance. As mentioned above, standard tests for decay resistance to fungi are destructive, laborious, long-lasting and really not suited to high-throughput phenotyping. Classical decay test on heartwood of 100 hybrid larch trees following standard CEN TS 15083 (16 weeks) but with only one fungus (*Coniophora puteana*) was firstly conducted to obtain mass loss percentages. An alternative using 2 mm thick boards cut from 4 mm diameter increment cores was tested where wood specimen were submitted to the fungus for 4 and 6 weeks. X-ray microdensitometry, before and after fungus attack, provided density losses at ring levels and mass losses at sample level. Mass losses between standard and the alternative methods were not correlated; as well only moderate correlations were found between mass losses and density losses of thin boards ($r < 0.48$). Decay test on thin boards proved so far not reliable. Therefore NIR spectrometry models were only developed based on data from standard decay tests and with spectra acquired on solid wood. For PLS regression, two levels of analysis were considered: sample and individual tree levels. At the block level, the best calibration model was characterized by a R_{CV}^2 of 0.65. At the tree level, the reference data were more accurate and consequently model quality was improved with best R_{CV}^2 of 0.80. These results confirm that NIRS is useful for evaluating wood durability, assessed with the new norm CEN TS 15083.

Perspectives for further increasing throughput capacity of NIR spectrometry can be seen in first attempts to use an imaging 2D-NIR Scanner, based on a hyperspectral camera, for characterization of spectral variations across surfaces and variations in chemical composition and other properties. The following figure illustrates results from use of a model for concentration of lignin obtained from scanning a SilviScan sample strip from pith (left) to bark (right) of Norway spruce. NIR spectra have been automatically collected from pixels of size 31 µm x 31 µm, covering all the surface of the sample. From each spectrum (pixel), a lignin content has been estimated, using a model developed in collaboration with IICT, where the reference analyses were performed using Pyrolysis GCMS.

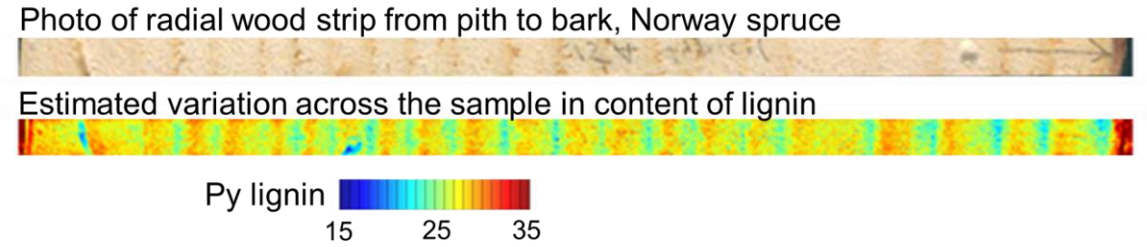

Figure 12. Illustration of estimated variation in lignin content across sample from pith to bark: the yellow-red colour represents early wood

Other properties like density and extractive content could be estimated from the same spectra, showing the high throughput potential of this approach to get between and within ring variation for one or several traits from a single scan.

Besides NIR spectrometry development, other non-destructive approaches were tested: as already mentioned above for decay resistance assessment from small wood specimen sawn from increment cores. Attempts to use diffractometry from SilviScan technology to assess spiral grain show some potential but it is far from being high-throughput. Acoustic velocity measured with a Fakopp was shown to be a good proxy to measure wood stiffness on standing young trees (3-4 years) with high enough correlations between young and adult trees. Finally, wood microstructure analysis of annual ring was further improved through development in microdensitometry of increment cores using X-ray CT scanning: that allows getting high-definition density and porosity profiles which are useful in several studies such as on environmental impact on wood formation.

Conclusions

Search for non-destructive, reliable, fast and cheap phenotyping methodologies is a priority in many fields of forestry research. Thanks to multi-disciplinary and inter-laboratories approaches, particularly visible around some joined experimentation (phenology, drought experimentation, decay resistance, NIR spectrometry models transferability), Trees4Future WP11 has succeeded in developing, improving or validating several high-throughput methodologies for a wide range of traits, particularly sensitive in a global change context. Large data bases are available; several scientific papers have been published or are being submitted and several others will be prepared in the coming months.

Some tools are usable as such and can be made available to the scientific community and industry. Some still need development but we showed their potential. Developing some new high-throughput phenotyping tools and methodologies is one thing; to consider their impact and their transferability is another issue. In this respect, we tried to answer to some of these concerns: for example, a key question for breeders is ‘does use of proxies for high-throughput phenotyping alter selection perspectives?’; to facilitate access of industry to NIR spectrometry developments, transferability of models to service labs was questioned.

Finally, the project allowed close collaboration among several teams which is expected to grow further beyond the project life. An invitation to settle down further a pan-European platform of NIR spectrometry for wood analysis has been launched.

1.3.4 Towards a collaborative development of tools for statistical analysis of forest genetic resources (Coordination: L.Sanchez, INRA)

1.3.4.1 Context and objectives

Statistical analyses are a key preliminary step in the assessment of natural genetic resources and breeding material in selection programs. In a context of rapid changes in demands, decisions concerning the management of these resources rely to some important extent on assessments derived from statistical analyses. The FP7 Trees4Future (T4F) project has made a significant effort in identifying, collecting and sharing relevant data for the scientific and stakeholder communities. This effort in data availability needs to be accompanied by appropriate analytical tools. Data, the basis of these analyses, is becoming increasingly complex as research advances, often incorporating heterogeneous sources of variation, multiple explanatory factors and experimental sites, or large and unbalanced pedigrees, to cite a few of the new raising complexities.

Navigating through this data complexity is not an easy matter for most analysts, and this starts already in the identification of adequate tools. Sometimes, the choice of statistical tools is somehow arbitrary and subjected to external constraints, including economic ones, rather than backed by technical reasons. Moreover, there are numerous statistical tools available, often comprising very specialized solutions, and there is no real consensus on the best statistical tool for the assessment of forest genetic resources. Although this richness in terms of solutions is indeed positive, it can easily become daunting for beginners, or lead to heterogeneity of choices among advanced users. Whether caused by the multiplicity of choices or not, tool-based barriers do exist among users of statistical tools in forestry, impairing discussions on common issues relating to the statistical analysis.

The development of the statistical package breedR in the framework of T4F aimed primarily at making available a comprehensive set of high performance statistical tools designed for the analysis of forest trials, with emphasis given to modelling several phenomena that, being of high relevance in forestry, were rarely available within a single tool. In order to foster interactions between users, the package was conceived with generality and openness as main drivers. The well-established R environment (R Core Team 2012) was chosen to be the natural environment for breedR, and its sources made publicly available for all potential developers.

1.3.4.2 Main scientific and technical results

What is breedR? breedR is an ensemble of different analytical modules that can be used interactively to construct models adapted to various experimental settings and underlying hypothesis. breedR works as an interface by which the user can set easily different analytical models, fit them and obtain results and diagnosis on the fitting. breedR builds up for the user all the equations and relies on external pre-compiled FORTRAN codes for the heaviest computations of model fitting. breedR fulfils the two main objectives of data analysis in forest tree genetics: assessing genetic and non-genetic variation at population levels and predicting individual performances for the different variation components. breedR was developed to be flexible for the user, and this is to be found at various levels. At the level of traits, by allowing the fitting of one or several of them, being temporally or spatially distributed, or simultaneously recorded on same samples. At the level of genetic factors, flexibility comes by allowing any pedigree to be considered no matter the complexity and depth, or by letting the user to include marker-based relationships and genetically-based competition. At the level of environmental factors, spatial statistics have been implemented to allow the user to compensate a posteriori for faulty experimental designs, together with the ability to account explicitly for genotype by environmental interactions.

At the core of breedR, we have a general mixed model framework. Basically, mixed models (MM) are statistical linear models that fit the observations in terms of fixed and random explanatory variables simultaneously (see box below). By their great flexibility, MM have become the workhorse in the estimation of breeding values in selected populations, where there can be a multiplicity of fixed and random explanatory variables like sex, age, site, block and family, to name a few of the more common variables. Both, the prediction of individual genetic values and the estimation of causal variance components at population levels can be dealt with within the general mixed model framework. REML (and Bayesian) estimates are used in the core system to avoid the problems caused by unbalanced designs, commonly found in old experimental trials. breedR proposes two alternative methods of inference for REML estimates, the EM ("expectation maximization") that is known to be robust although slow converging, and the AI ("average information"), which is faster but at the cost of some sensibility to initial values and difficulties with some complex models. MM calculations are performed by a series of FORTRAN programs in the background (Misztal 2008). These are well-known by their efficiency in dealing with complex models and huge datasets, notably in animal breeding.

Thanks to MM flexibility, different models were developed to account for specific problems, some of which came as a novelty in the context of forest genetics. Our development plan comprised three of these novel formulations. In all three, the basic properties of these methodologies are already well known and applied in the context of non-forest species but their practical application is a fairly recent phenomenon in forest biometry. Below, we describe briefly their main features in the release v0.11.

Competition models (CM): Competition reflects the impairing interplay of closely growing trees, often when resources are limiting. Competition can impact the performances of neighboring individuals in such a way that variation among individuals may result substantially distorted from what is predicted following a purely non-interactive model. Competition is known to be relevant in forest trials typically at the age of phenotypic evaluation. Often, while its effects are acknowledged by breeders, it is not accounted for in the evaluation process. To account for competition in breedR, we followed a MM approach described in detail in Cappa et al (2015). This approach provides predictions for both direct breeding values and competition genetic effects, as well as estimates of the corresponding genetic correlation between direct and competition effects. For fitting this model, trees layout in the field needs to be recorded by spatial coordinates, which is a common feature in most experimental trials. The user can modulate the decay of the competition effect with distance to fit conveniently biological and trial specificities. There is also the possibility to include a specific residual for competition, which could be of relevance in cases where the phenomenon has, at least in part, non-genetic causes.

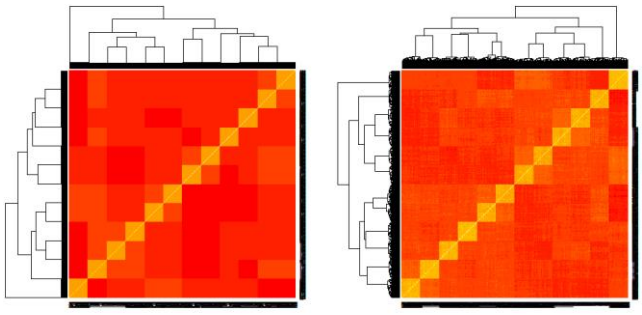

Figure 13. Relationship matrices that can be used in breedR, default pedigree-based (A), marker-based matrix (G). Lighter colours denote higher values, like those in the diagonal. While A provides a limited number of relationship classes, G provides a quantitative distribution of relatedness, even at the level of single cohorts. This denotes the ability of markers to capture relatedness differences, which can be used to improve predictability of breeding values.

Pedigree-free evaluation models (PFEM): Whenever the relationships between individuals are not known with certainty or not recorded in pedigrees, like in the case of wild populations, other alternative approaches can be implemented instead. In these alternatives, the resemblance is inferred at DNA level by the use polymorphic genetic markers. This marker-based resemblance can be used in the MM framework to infer genetic parameters of the population under study in a similar manner as in pedigreed populations (Kruuk 2004, Frentiu et al 2008, Sillanpää 2011). This is of special relevance for forest trees for two main reasons: a) because most of the species of interest for forestry can still be considered as undomesticated, which makes the study of wild populations of great relevance, notably when considering the breath of their genetic diversity and distribution; and b) because trees are large living organisms with lengthy maturation and that occupy considerable space, which means that the establishment of experimental trials is costly or even unattainable when evaluations are to be made in a short timeframe. Additionally, the rapid advances in genomic resources have made possible, even for non-model species, access to a large number of genetic markers, which are required for precise inferences of marker-based resemblance. Yet, few are the examples where these approaches are applied to natural populations of trees. In breedR, there is the possibility to include a marker-based resemblance matrix (G) to be used instead of the classical pedigree-based additive relationship matrix (A), and to model the covariance between individual genetic effects. This feature is part of a generic module that allows the user to use any arbitrary incidence and co-variance matrices to feed random effects in a MM, which adds great flexibility in the formulation of specific models.

Figure 14. Maps of spatial effects in a Douglas-fir progeny trial as resulting from a model with Blocks design (left), Splines and Autoregressive residuals (AR, right). Red graduation denotes favorable environments for growth, while blue colors indicate the unfavorable zones in the trial. While Block model provide the same block effect for all individuals appertaining to a given block, the other two methods give finer detail of what is happening within blocks.

Spatial statistics (SS): Experimental trials in forestry often cover large and heterogeneous environments. This heterogeneity is at least partially accounted for by devising a priori the experimental design with random blocks. This is the classical way of addressing the environmental component, but there are alternatives where heterogeneity is accounted for a posteriori, by analysis. These alternatives have the advantage of being flexible and adaptable whenever there is a faulty design in the trial, either by conception or by ageing. breedR is able to accommodate two alternative a posteriori models to address heterogeneous environments in trials, apart from classical block models. Both alternative models provide individual predictions for environmental effects, allowing the user to obtain detailed maps of the environmental heterogeneity down to single trees.

The first of these two alternatives is the autoregressive residual modelling (AR), which consists in partitioning residuals into a spatially dependent effect and an independent residual. The autoregressive term is modelled as a product of autoregressive correlation matrices for rows and columns. It follows a well-known methodology, described in detail in Dutkowski et al (2006). AR requires spatial coordinates with regular spacing in the trial. breedR provides estimates of the correlations between neighbors within rows and within columns, as well as a spatial variance component. The second alternative is based on the use of B-splines to model the environmental heterogeneity. The methodology is described in Cappa and Cantet (2007), and basically consists in using a number of overlapping B-spline functions in the two dimensions across the trial, and scale them by coefficients that will be estimated. The output is a smooth continuous surface representing the environmental heterogeneity in the trial, resulting from summing up the overlapping scaled B-spline functions. breedR provides tools for diagnosis of spatial heterogeneity based on several kinds of variograms. Although AR and B-splines are well known, with an abundant literature, and generally lead to significant improvements in the estimation of the genetic components, their use in forestry remains somewhat reduced. This is probably due to their conceptual complexity. In any case, most phenotypic evaluations in experimental trials could potentially benefit from spatial adjustments, delivering records that are less prone to bias by uncontrolled or hidden environmental factors.

G×E analysis: Unlike previous procedures, which can be qualified as novelties, the family of G×E analyses are routinely implemented in plant sciences, notably for annual crops. As there is an increasing interest in the assessment of phenotypic plasticity in forest trees, notably in the context of global climatic changes, it was fully justified to offer the possibility of G×E analyses in breedR. Given the flexibility of MM, G×E terms can readily be included without the use of a particular module.

Parallel to development, there has been an effort to provide tutorials on the different features of breedR. These tutorials are downloadable documents, accompanied by R scripts for the user to try the contents of the demonstration documents. Some of the features covered by tutorials were developed as a demand from the beta-testing community, and cover aspects like: additive genetic models in mixed populations, general and specific combining abilities, handling pedigrees, estimation of heritabilities and standard errors, missing values, to cite some of them. Training has been an essential part of the dissemination of the tool, as well as invaluable for fostering exchanges with and among users. Training was organized in the form of workshops with short conceptual presentations and practical exercises with real and simulated data. The contents of the workshops including tutorials, presentations and data are available at the breedR portal (for the Spanish course, http://famuvie.github.io/breedR/workshop/). Support is provided through the Github forge, where users can post directly their issues during beta-testing, and also via a mailing list breedr@googlegroups.com.

The users' community was initially composed by 12 partners of the T4F consortium, which were also formally the beta-testers of breedR developments. This initial community of approximately 20 people was particularly active during the training events and afterwards at the final stage of T4F, providing a valuable feedback to the development team at INRA. At the time of writing this report, approximately 100 people have reported using breedR for their analyses, and keep to some degree a contact with the development team and the rest of the community.

1.3.5 Modelling of spatial deployment of species/provenances and of forest resources (Coordination: D.Ray, FR; G-J.Nabuurs, Alterra)

1.3.5.1 Context and objectives

The overall aim was to integrate aspects of genetics, provenance knowledge and wood quality into large scale modelling tools, and to improve the infrastructure and compatibility of these tools to assess goods and services, sustainability, and mitigation and adaptation strategies for European forests. Sub objectives are:

- Assess requirements for compatibility and exchange of data between genetics and wood quality on the one hand and large scale assessment models on the other hand.
- Improve infrastructure and performance of three existing European models to ensure compatibility between models and the databases compiled by the current project
- Develop an open and user-friendly structure supporting the use of these models by the wider European research community to assess goods and services, sustainability, and adaptation and mitigation capacity of European forests.

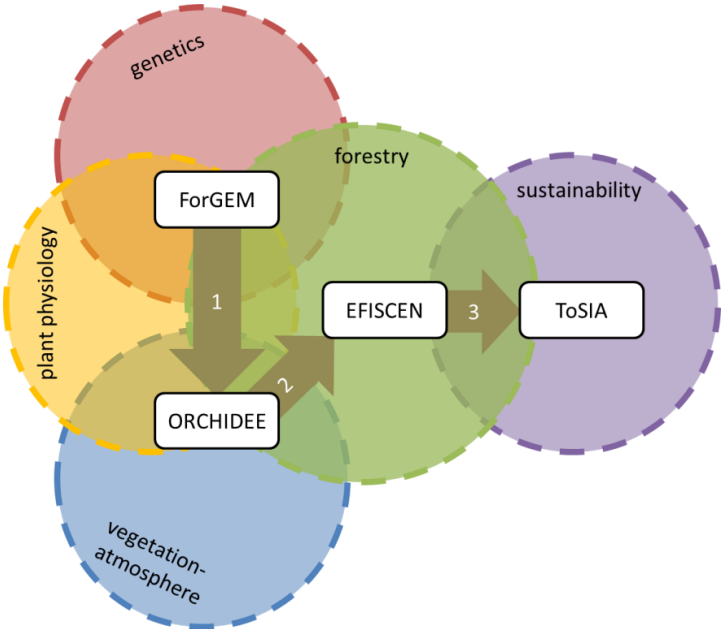

Figure 15. Conceptual coupling of models

In addition, Trees4Future looked into approaches to analyse Europe-scale provenance trials of two tree species, to assess the suitability of provenances (and origins) for tree breeding, and the deployment zones where the material might be most effective in the future European climate. We also developed a web-based climate matching tool to help: forest policy makers, tree-breeders, tree nursery managers, forest planners, forest managers and forest owners, understand potential abiotic consequences of projected climate change.

1.3.5.2 Main scientific and technical results

From the respective focus of the different models we designed the model chain as illustrated in Figure 15. Although covering forestry topics, each model has its own focus (Table III). ForGEM informs ORCHIDEE on the spatial heterogeneity in species performance (1). As plant physiology-based model ORCHIDEE is sensitive to climatic forcing, as such it can inform EFISCEN on the differential effect of climate change on the growth and development of different tree species (2). Finally, ToSIA needs information on forest resources and harvested timber under different management and climate scenarios, as well as other variables (e.g. carbon in harvested wood, dead wood amounts etc) (3). Next to these couplings each model can use data generated by other models not mentioned in the chain.

Model input-output exchange

To practically couple the four models, parameters have been identified that can be exchanged between models (Table III). The coupling focused on the following aspects:

1. The spatial differentiation of species specific parameters for photosynthesis as ForGEM would calculate their locally adapted equilibrium value are used in ORCHIDEE as functional group specific parameter values.
2. The relative change in NPP per functional group and location between a specific scenario and the baseline scenario are used as relative change in species specific growth-rate in EFISCEN. This change in growth-rate can be derived from continental scale wall-to-wall simulations as are done in ORCHIDEE (2), or from simulations with stand-level models (e.g., ForGEM) at many locations (2a).
3. The projected harvestable timber-volumes by EFISCEN are used in ToSIA as input.

Each of these couplings brings about challenges and opportunities. Coupling 2 and 3 have been tried and tested in previous studies. For these couplings, tools are developed to automatize the exchange of parameters/information between models. Coupling 1 is new and thus the discussion on this coupling is more conceptual. The coupling are summarised in Table III and described in detail below.

Table III. Summary of model couplings

Name model	*Dependent on*	*Means of exchange*	*Parameters used from other model*
ORCHIDEE	ForGEM	manual manipulation of input file	Maximum rate of carboxylation (µmol $m^{-2}s^{-1}$) and Maximum rate of Rubp regeneration (µmol $m^{-2}s^{-1}$)
EFISCEN	ORCHIDEE	Look-up tables and R scripts	Relative Net Primary Production (%)
ToSIA	EFISCEN	EFISCEN output database and mapping tool	forest area (ha), roundwood removals (m^3)

Provenance performance of Douglas-fir and European beech in Europe

Our method linked climatic niche models to classify provenance and origin zones. Douglas-fir provenance zones are shown in Figure 16, European beech provenance zones are shown in Figure 17.

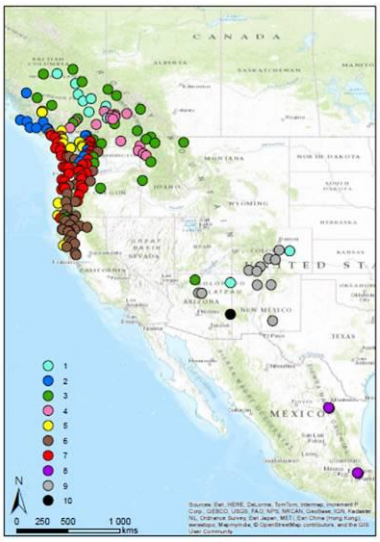

Figure 16. Ten provenance regions characterised by correlative niche modelling using 14 climatic variables from WorldClim

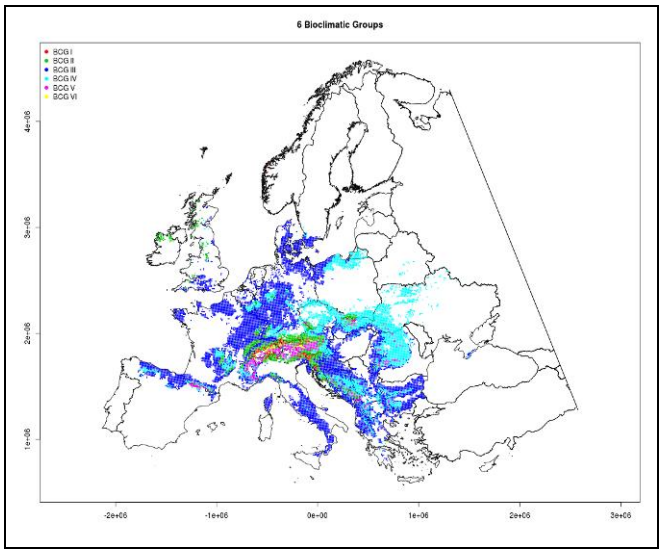

Figure 17. European beech provenance regions defined using bioclimatic zones in a cluster analysis

Previous IUFRO (Douglas-fir) and COST Action (European beech) trials had tested seed origins within provenance zones across Europe at numerous trial sites. Our analysis provided the following results for Douglas-fir (Table IV), and European beech (Table V). These are recommendations for the use of seed origins of Douglas-fir and European beech for tree-breeding and use in different regions of Europe. We also recommend that future provenance trials are designed as 'common-garden' trials and tested to the limits of the potential niche for the species. The work provides a template methodology for future researchers to evaluate provenance or other tree materials to site conditions.

Table IV. Superior quality Douglas-fir phenotypes tested in Europe and scored on height growth and survival

Bioclimatic Zone	Location of bioclimatic zone	IUFRO Seed Origin(s) (Name) # sites performance superior/# of sites tested	Recommendation for use in European regions in future
6	Willamette Valley and Klamath Mountains - Oregon	1113 (Mill City) - average 86% chance of class 1 or 2 performance from 12/14 test sites 1115 (Corvallis) – average 73% chance of class 1 or 2 performance from 11/15 test sites	Fairly good growth and survival in Ireland, UK, Normandy, Galicia, and southern Europe
5	Puget Sound and Coastal Oregon	1057 (Granite Falls) – average 82% chance of class 1 or 2 performance om 9/11 test sites	Excellent growth and average survival in France – good growth and survival in Ireland, UK, southern Europe, Poland, Baltic States, Turkey – Average growth and survival in central Europe

7	Coastal and Cascade Ranges - Washington and Oregon	1086 (Naselle) – average 85% chance of class 1 or 2 perfomance from 13/16 test sites	Excellent growth and average survival in Ireland, UK, central Europe, southern Europe, Norway, Normandy

Table V. Superior quality beech phenotypes tested in Europe and scored on height growth and survival

Bioclimatic Zone	Location of bioclimatic zone	European beech database seed book number	Recommendation for use in European regions in future
2	Foothill areas of mountains and hills in Ireland, Scotland, England, Germany, Switzerland, Austria, Slovenia and Croatia	8775	Scotland, Norway
3	Large areas of central and southern (at higher elevation) Europe	8776 - 8781 - 8783 - 8869 - 9111 – 9114 - 8771	Germany, northern France, Poland, England, north central Europe
4	Lowland central Europe	8761 - 8784 - 8785 - 8877 - 9168 -8762	Poland, Baltic States, Ukraine

1.3.6 Networking infrastructures and experts for forest research (Coordination: J.Climent, INIA; B.Heinze, BFW; L.E.Pâques, INRA)

1.3.6.1 Context and objectives

Many groups in Europe are conducting forest research. Different subjects are tackled, and the emphasis varies among groups – some are more interested in generating basic knowledge, others are keen to apply new findings in practice. Perhaps nowhere else, the direct link between basic research and practical outcomes is as clear as in many forestry-related fields. Scientific findings often feed directly into management plans and action. Disconnected, fragmented research efforts may therefore produce heterogeneous management regimes for forest in Europe, even if the climatic and soil conditions are often homogeneous over large areas. Thematic research networks developed in this project are therefore linking scientists with interest in similar topics: from phenotypic plasticity to phenology and social implications of forest breeding. Within these networks, the current state of knowledge is discussed and summarized, in order to have a solid base for developing the field further: what are the next challenges? How can these be tackled, given the opportunities that joint use of research infrastructure and expertise can provide?

In the field of forest reproductive material, the great time lap between planting and timber harvest provides such a challenge. Use of the best suitable plant material is needed for producing stable, productive forests; yet the quality of planted forests is often only visible after years, or even decades. Genetic tests developed and harmonized jointly among project partners are needed in order to assess and certify nursery stock, beyond the paper documentation now in place.

A little number of laboratories in Europe (BOKU-AT, CIRAD-FR, CNR-IVALSA-IT, INNVENTIA-SE, INRA-FR, IICT→ISA-PT, LUKE-FI) are equipped with NIR spectrometer

equipment and have developed expertise on its application to forest wood products. According to their research environment, they have developed several calibration models for species and wood properties related to their interest and needs. Some of them have been published in academic journals, some not, so that many of these models remain confidential and not accessible to the research community and industry.

1.3.6.2 Main scientific and technical results

The PhenoPlastFor network: Phenotypic plasticity is a trendy topic in forest science in general and in tree breeding in particular. The PhenoPlastFor thematic network implemented in Trees4Future has allowed clarifying several misunderstandings about the concept and how to apply it in practical forestry. By compiling updated literature, asking a group of experts and surveying experiment sets, it is now clearer what we can and what we cannot obtain from extent breeding experiments. In particular, obtaining heritability values of the plasticity for key traits is extremely difficult, except if plasticity is determined in a time-dependent basis, thanks to the retrospective character of dendro-chronological methods or densely-repeated measures across time. In these cases, extracting the ontogenetic signal is a common problem. A review paper is on the way, using a selected group of case studies to discuss some of the key questions, with a strong emphasis in the applicability of the results and conclusions.

Towards the development of a molecular platform for fingerprinting and traceability of biological material: Powerful genetic tests have been described for several forest tree species. These tests facilitate the investigation of individual relationships among plants, just like among parents and children. Yet, it is often unclear in detail where the ‘parents’ of a seed lot should be searched for: forest reproductive material (seeds and plants) are traded under designations of ‘regions of provenance’, which may cover comparatively large geographical areas. In other cases, e.g. in some conifer species, seeds are collected at the time of timber harvest, from felled trees that cannot serve as reference material any longer. The investigation and description of regional genetic variation and variants are therefore a necessary basis for any genetic comparisons, and for seed and plant lot identification and certification. Certification schemes are necessary because they lay down uniform and generally accepted rules and assure their implementation with acceptable input. Forest reproductive material may ‘change hands’ quite often between the time of seed harvest, and the selling of plants from the final nursery. There are often many intermediate steps involving the trade and storage of seeds and plants. ‘Traceability’ describes the extent to which such steps in the market are checked for plausibility: is the plant material sold to a forest owner still genetically identical to the seed originally harvested in a selected forest, sometimes years ago? Paper documentation can supply this data, but scientific analyses are often desired as a back-up. Traceability systems strengthen confidence between producers (e.g. seed harvest companies, forest nurseries) and consumers (e.g. forest owners) and lay down the responsibilities of suppliers and buyers at different stages of the supply chain. This is quite common in the food product market, but relatively new in forestry. Desirable features of forest reproductive material traceability systems would be the identification of correct species (which can be an issue for many similar-looking forest tree species in nurseries) and to connect this material to its source, i.e. the seed stand or region of provenance.

Project partners have summarized and evaluated the state of knowledge regarding genetic markers and regional data at the start of the project, concentrating on the main conifer and hardwood species in Europe.

For some of the seven tree species (*Fagus sylvatica , Quercus robur* and *Qu. petraea, Prunus avium, Picea abies, Larix europaea, Populus sp.*), genetic landscape maps have been published, though they

are still rather coarse (e.g. for beech, oak, spruce), while in other species, such basic information is still lacking largely (e.g. cherry, pine, larch). The genetic information contained in a particular compartment of plant cells called chloroplasts (which convert sunlight to chemical energy) is currently the most suitable genetic tool to trace material back to particular regions, if such genetic maps exist. Therefore, this marker system should be further developed to increase spatial resolution across Europe. Additionally, this type of DNA is useful for less optimal material, such as timber that has been cut some time ago. Experience with the use of a particularly useful type of genetic markers, so-called microsatellite or simple sequence repeat (SSR) markers, was exchanged and critically compared. For some of the species, clones are traded (e.g. in *Populus* and cherry), and here, the genetic fingerprints obtained from these markers are ideal means of identification. Different marker sets are currently in use, but the resolution that they provide is comparable across laboratories. Some standardization of methods among laboratories is desirable in order to avoid divergent interpretations; though the use of several diverse markers should rule out such outcomes. Many such sets can now be run in a single lab reaction, which saves on time and resources, without compromising data quality. Fresh material (from seed, seedlings, or trees) can be traced back to the stand of origin, if it still exists. Statistical tools are important in this respect, as it is not always possible to directly infer the parents of each seed or plant in the stand.

There are examples of successful integration of genetic markers into checks of conformity of labels and genetic quality in the market, but they require additional measures. For instance, in Germany, voluntary certification schemes work on the basis of physical reference samples taken at different steps of the forest plant production chain. These are tested depending on a statistical sampling scheme in designated laboratories. The reference samples and laboratory investigations also come into play when any doubts arise about the identity of seeds or plants.The most apparent gaps in such knowledge were identified.

Towards a pan-European NIR spectrometry platform for wood properties analysis

A first achievement was to survey and compile all of these models (existing or in development). They cover at least 9 groups of species (*Eucalyptus, Populus, Liquidambar, Pterocarpus, Quercus, Robinia*) and (*Larix, Picea, Pinus*) and several categories of traits (Extractives, Lignin, Cellulose, Hemicellulose, sugar, S/G, fiber length, pulp yield, density, shrinkage, MOE, MOR, mass loss, hydraulic properties), which show the broad application of NIRS. A leaflet has been produced to disseminate this information broadly with the hope to improve accessibility, use and new development of these predictive tools.

The second achievement was to test the transferability of NIR spectrometry models from lab to lab. The idea behind was in the option to have established a service lab – which could routinely conduct analysis for its clients (industry, breeders, etc)- to build on already existing models. As a reminder, construction of operational models imposes i) to have access to large sets of samples covering well the variability of the property studied, ii) to get reference data on the property based for example on standards; iii) to have the analytical tools and expertise to build on the models, and iv) to validate developed models. Based on a round robin test among 5 laboratories using various NIR spectrometry equipment and analytical methods (for prediction of lignin content in Norway spruce samples), different calibration transfer algorithms were tested. Some proved reliable for an adequate transfer of models.

A last objective is to facilitate exchanges of expertise and of scientific (analytical procedures) and technical (conditioning of samples, equipment) information as well as of wood material among these labs. Strengthened collaboration and innovative developments are expected.

1.3.7 Improved access to forestry research infrastructures (Coordination: F. Miglietta, FEM/CNR)

1.3.7.1 Context and objectives

Trees4Future aimed to support forest research and help the European forestry and wood industries develop sustainable solutions for the future in the context of climate change, by improving and facilitating access to state-of-the-art research facilities throughout Europe. These include databanks, biobanks, models and decision-support systems, laboratories and other facilities. The Transnational Access programme offered external users free access to the 28 Trees4Future specialised research structures and facilities:

Genetics, Genomics and Tree Breeding

Trees4Future offered access to a wide range of facilities on genetics, genomics and tree breeding: from comprehensive on-line databases such as the EVOLTREE data warehouse and the Treebreedex databases, to state-of-the art laboratory facilities e.g. on genotyping and vegetative propagation.

(Eco-)Physiology and Biotechnology

Trees4Future offered access to versatile facilities for analyses on functional ecology, tree-water relations, dendrochronology as well as root analysis.

Wood Science and Technology

Trees4Future offered access to top class laboratories for analyses of wood properties and tree physiology, as well as access to state-of-the-art equipment, such as the Nanowood scanner by the University of Ghent, and the SilviScan equipment for measurement of wood and fiber properties by Innventia.

Models and Data Analysis

Trees4Future offered access to a range of decision-support tools for making prognoses on the development of Europe's forests, assessing the sustainability of their management, and analyzing the impacts of climate change.

1.3.7.2 Main scientific and technical results

The activity of the Transnational Access of T4F was very intense and successful especially in the last period, and the 6-month-extension allowed to significantly increase the number of applications processed. Changes applied to the procedure according to decisions taken by the Executive Committee helped to implement a practice that has worked out to be right and helpful, not only to applicants but also to Site Managers and reviewers. In its lifetime, the TA Program went through different phases, from the initial weakness in numbers and speed, to the final growth and efficiency. The main “tool” developed in these 4 years and a half, which was both a practice and a result of joint efforts, was the collaboration and interaction with all actors of the program – Site Managers, applicants, reviewers – and with the T4F Team in charge of communication.

The Call for Access was written in collaboration between the TA Team and the Project Management and the Call was launched in June 2012. Rules and procedure were re-written in a user-friendly way by WP4, the Team in charge of dissemination and communication, and published on the website.

The TA Team collaborated in promoting the existence of the program using a database of contacts and sending out messages to researchers and institutes. At the initial stage of the project it was

important to inform people of the existence of the possibility to get support for the use infrastructures which were either not accessible or accessible only upon payment. All partners of the consortium were asked to be active in this stage, promoting the Call among their contacts and partners. Despite of that, a low number of applications was received in the first months (19 till the end of the year). The first 2 visits took place in November 2012 but we had to wait till March and April 2013 to have other users at work. This means, the promotional role of users was delayed and its effect could be seen only later on. Using the contacts provided by users in their User Group Questionnaire, we could send out several promotional messages to invite people to apply to the program. This method proved to work, even if numbers were not so high: we received around 10 applications thanks to our invitation. A great promotional role was played by users themselves, and thanks to the collaboration of WP4 we were able to amplify this promotional effect publishing their comments on the website. The constant update of the webpage using information and answers collected through the post-visit questionnaire made the site a useful instrument of information and promotion. The team of WP4 developed a very successful communication, adding to the description of site the comments received by users.

A visual instrument to promote the Call on the website and to update the situation during the lifetime of the project was developed. A map was periodically updated, showing visited labs and working countries of applicants. Each point on the map could give the internet user information about the title of the project, the institute where applicants were working and the duration of stay at the lab with the support of T4F. A peak in submissions between November and December 2014 was recorded International meetings have proved to be the best promotional instrument: people meet in person, questions and answers can be done very smoothly and efficiently.

People interested in the Transnational Access program were invited to go through a two-stage application procedure; they were advised to interact with Site Manager before submitting the proposal, but this was not compulsory. The pre-application form was a two-page form to be filled with a short description of the project and main data of the applicant. We had to check the eligibility of the applicant according to the transnational criterium: no matter the nationality, he/she had to be working in one of the eligibile countries (EU and Associated Countries) different from the country of the lab that she/he wanted to visit. Another criterium determining eligibility was the belonging of the applicant to institutes/institutions/working units external to the consortium. After checking the eligibility of the applicant, we sent the form to the Site Manager (or Sites Managers) for their feedback: he/she/they had to evaluate the feasibility of the project according to the scientific value, to the availability of time and infrastructures at their site. At this stage, it was recommended to Site Manager and applicant to get in touch, in order to exchange comments and suggestions on the project. If the Site Manager's feedback was positive, applicants were invited to submit the second-stage application form (or “full application”), a more detailed description of aims, objectives, scientific procedure, plans for publications, etc. This full application was submitted to 2 external reviewers; if they differed in their evaluation, we either had a 3rd reviewer or the Executive Board deciding on the approval. In many cases, reviewers suggested amendments and changes that prolonged the reviewing procedure once applied.

The peer-review procedures

Trans-National Access involved a careful assessment of each project, based on peer-review. A User Selection Panel was created, composed by scientists selected according to their expertise in the field and to their experience as scientific reviewers. In the preparation phase of the Call for Access we asked Site Managers and T4F partners to provide a list of candidates. We then sent out invitations in order to collect availabilities. Out of 57 scientists contacted, 53 confirmed their availability to

collaborate in the reviewing procedure. This initial confirmation was a kind of generic promise, to be confirmed each time during the 4 and a half years of the Project. In the first months of activity, we had great difficulties in receiving prompt answers from the contacted reviewer: our program was new to them and the unpersonal request of someone they did not know made things harder. In fact, the first reviewing procedures lasted several weeks. In the following period, however, we had less difficulties in getting quick answers from reviewers: the previous activity and the fact that Trees4Future was at least known to them, simplified the issue a lot. In the evaluation procedure, not only were reviewers asked give their "yes" or "no" to the project, but they were invited to give comments, suggestions and hints for an improvement of the proposal. In many cases, reviewers who suggested changes were also available to evaluate the amended project, and this strongly improved the quality of the final approved submission. In some cases, Site Managers got directly involved in the procedure, helping the applicant to answer the reviewer's questions and doubts, and explaining some open issues.

Summary of applications and approved projects

A. Pre-applications

From June 2012 to October 2015 a total number of 162 pre-applications was received. The trend was quite steady in the first two years of the Program, with some peaks corresponding to promotional activities at international conferences and to the final deadline fixed for the end of October 2015. 19 applications were received in 2012, 34 in 2013, 32 in 2014 and 77 from January to October 2015, with 24 only in the month of October. The average number of applications per month is over 10, with a peak in April 2015 and another peak in October 2015: during the last year of opening of the calls for access we received as many applications as in the previous two years. The most requested field was genetics and genomics, with ASP in Germany, BFW in Austria and INRA GTF in France hosting altogether 34 projects for visits that used almost 800 working days. However, a positive trend was noticed for sites active in the field of Wood Science, like the Nanowood Scanner of University of Ghent in Belgium, which in the end hosted 14 projects.

B. Approved projects

The total amount of approved projects was 114, with 3 visits cancelled in the last minute. All of these 111 accesses (either personal visit or remote access with sending of the samples) took place until the end of April 2016. Most applicants used one single infrastructure and only a few applied for so called "split visits" (one lab visited twice) and for "multiple visits" (one project which used 2 or more labs). We also had "continuation projects": after some months applicant and Site Manager agreed on the continuation of sampling and working at the site. This kind of projects (just 2) went through a simplified reviewing procedure, which involved the Executive Committee. Though in the first half of the project lifetime it seemed that most applications were aimed at infrastructrures in the field of genetics, the final report shows a balanced situation between the field of genetics (51 accesses) and the field of Wood Science (47 accesses). This increase since the end of 2014 is certainly due to the promotion activity made at scientific conferences and meetings. The least requested field was Ecophysiology, with only one partner asked to provide access (IDPAN labs in Poland), while Models and Data Analysis hosted 19 users.

C. The users

Applicants' working countries do cover most of Europe. There is still a major request coming from Spain, with 21 group leaders based in Spanish institutes, but the initial difference noticed in the first two years of the project between South and North Europe slowly disappeared. There were in total 10 approved projects from Germany, 6 of them submitted after November 2014; from the 8 applications

from Sweden, 5 were submitted in the last year; from the 3 applications from Great Britain, 2 were added after November 2014. In this last period, we also received submissions from Turkey and Slovenia.

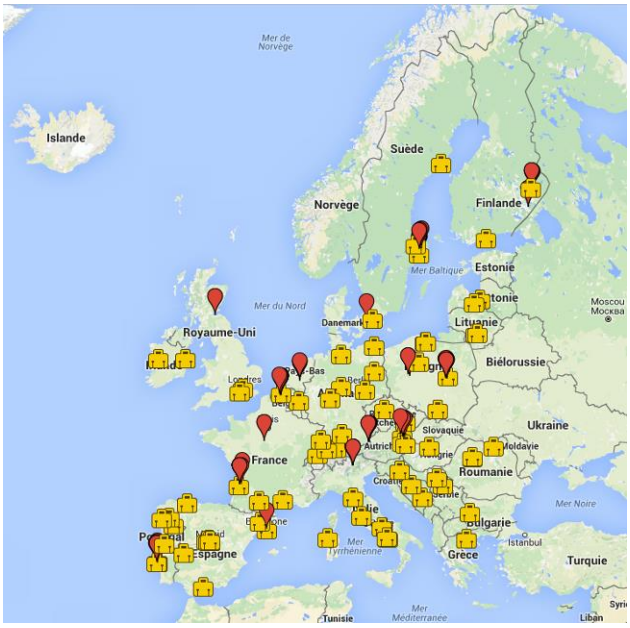

Figure 18. Infrastructures offered by TNA programme (in red) and countries of origin of visitors (in yellow)

D. The site managers

One of the main tasks of the TA Team which was developed and improved was the tight connection with Site Managers. This proved to be an important issue which was particularly stressed during the third annual meeting that took place in Tulln, Austria in November 2014, where first results could be achieved: Site Managers are the face and body of Trees4Future, while the TA Team is simply a faceless voice. The support and collaboration in this direction were developed in the different stages of every application: in many cases Site Managers and applicants were in touch since the beginning of the procedure, also in the stage of writing the pre-application; in other cases we played an intermediation role, helping Site Managers to answer administrative questions. Some support was asked by Site Managers in the reporting phase, in order to double-check number of units provided for each visit. In fact, the TA Team kept track of the whole procedure, from pre-application to reimbursement, including double-checking access units and days spent at the hosting lab.

1.3.8 Strategic Innovation and Research Agenda and European Tree Breeding Centre (Coordination: J.van Brusselen, EFI)

The Ministers for Agriculture and Forestry of the EU member states proposed a budget of more than 5 billion Euros for the implementation of the Forest Strategy until 2020 (including Research, Technology Development and Innovation – RTDI).

The forestry research community should set the goal of at least 500 million Euros of that budget to be invested into RTDI. Therefore this agenda is published in the right time and can help advocating where the EU should direct strategic RTDI support for the best result.

1.3.8.1 Context and objectives

A key project objective was to establish **interactions with and among the different stakeholder groups including industry, professional community, policy makers and public** at large, through Trees4Future interactive workshops and panel discussions. The aim of the interactions was to disseminate knowledge of the Trees4Future activities and results, and to map long-term expectations towards RTDI and related infrastructure development, of relevance to industries, society and RTDI actors.

Trees4Future also aimed a **long-lasting integrated research infrastructure**, enabling access and utilisation of the project results also beyond the project duration, thus contributing to the Europe 2020 strategy and the European Research Area - ERA. This required the development of a strategic innovation and research agenda, the development of a roadmap to achieve sustainability of Trees4Future results.

1.3.8.2 Main scientific and technical results

Foresight methods i.e. investigation of alternative futures in a long time horizon were applied in mapping out research needs together with the stakeholders (both through workshops for collaborative learning, and data and information collection through internet survey tools) – The foresight approach provides the Trees4Future with input on trends and drivers, possible change factors and emerging issues what comes to e.g. long-term developments of the markets at global level, needs and demands of future societies, technology developments which affect different end uses, new innovations underway, infrastructures and RTD needed to achieve needed innovations, etc..

A **Strategic Innovation and Research Agenda (SIRA)** was developed based on interactions with the various stakeholder groups, a report defining research needs identified within Trees4Future, aiming to match future forest research activities more closely with environmental, economic, industrial and societal needs.

The SIRA is based on two main inputs: firstly on a survey that was widely disseminated amongst the research community and secondly on interviews with stakeholders that were performed as part of work package 3, and which included a short section specifically incorporated to collect stakeholder inputs for the SIRA.

The strategic innovation and research agenda (SIRA) identifies key themes, key possibilities to cooperate and potential funding sources (European, transnational, national and regional funding) to which the implementation of this the agenda would be of high relevance.

Key guidance in the strategic innovation and research agenda development process was sought from:

- key objectives of Trees4Future, tree breeding, forestry research
- expectations of practitioners and stakeholders
- consideration of what research efforts should be enhanced

The material collected throughout the various stakeholder panel discussions, workshop and consultations formed the basis for a policy brief. The SIRA brief was published for a wide

distribution as the "Trees4Future Strategic Innovation and Research Agenda". The SIRA was distributed to all project partners as hardcopy and to consultation participants in digital format, encouraging all to use the agenda as a tool in their research advocacy activities, and to share the agenda with their regional, national and European stakeholders and peers.

A copy of the SIRA can be downloaded from:

http://www.trees4future.eu/uploads/EFI_Trees4Future_SIRA_06062016.pdf

A list of acknowledged contributors to the SIRA development process can be found from:

http://www.trees4future.eu/sira.html

A **roadmap for sustainable availability and exploitation of Trees4Future results** was designed. Trees4future engages in dialogue with key identified forest sector stakeholder groups in order to develop and communicate a strategic research agenda focusing on challenges such as improved and sustainable production methods, optimal use of forest resources to various industrial and societal needs and mitigation of climate change. Some of the key issues that were addressed in the roadmap are listed here below:

- ***Sustainable research infrastructure: databases, clearinghouse, models, tools and training materials:***
  - the T4F website is being kept operational for at least the five coming years and then re-evaluated based on web-use statistics;
  - the T4F Clearinghouse, uses a self-updating architecture. The practical implication is that if changes are made to the data sources registered with the Clearinghouse, the Clearinghouse automatically becomes aware of these changes. This design means that the service will not lose value due to a lack of active maintenance;
  - the Climate Matching Tool will be kept operational in connection with the T4F website
  - free and long term availability of the EFISCEN software has been accomplished by releasing the EFISCEN source code as open source under the GNU GPL licence. This allows for the public availability of the tool, and further development by any interested party, via https://github.com/EuropeanForestInstitute/efiscen
  - T4F databases and the search interface for genetic data:
  - BreedR: the suite of statistical modules for genetic data analysis, was written on purpose as R open-source codes which can be freely downloaded at http://famuvie.github.io/breedR/
  - training materials that were developed in the project, are uploaded to and remain accessible through the Trees4Future webportal (http://www.trees4future.eu
- ***Advocacy: SIRA*** *(as elaborated upon in the above text)*
- ***Expertise networks:*** T4F colleagues' contact details remain accessible in the T4F intranet site, and over 130 scientists were invited to a dedicated LinkedIn group.
- ***T4F service portfolio:*** In absence of project funding the Transnational Access programme can no longer offer external users free access to the 28 Trees4Future specialised research structures and facilities. However the TNA service portfolio will keep the structures and facilities visible via the website (http://www.trees4future.eu/transnational-accesses.html) to

further encourage cooperation within and outside the consortium. Infrastructure holders were asked for their readiness to keep infrastructures accessible to paying customers. There were no explicit objections to this request and contact infos were updated for TNAs where this was necessary. None of the TNA providers agreed to publish TNA cost information on the website, with the argument that this needs to be considered on a case-by-case basis. Interested parties are therefore invited (via the individual TNA description pages) to contact the infrastructure holders directly should they wish to make use of the infrastructures.

- ***European Tree Breeding centre***

Thanks to FP6 and FP7 EU programs for Research Infrastructures Networks, European breeders together with scientists from other disciplines could start settling down scientific and technical basis for **a future European Tree Breeding Centre**. The TREEBREEDEX project (coordination action, 2006-2011) aimed primarily to network European breeders' and forest geneticists' teams and to survey the status of breeding progress across Europe, of scientific knowledge on the genetics of major forest tree species, of skills and facilities devoted to breeding, and to identify research gaps and needs. Since their launching, breeding programs have gathered huge genetic resources (breeding populations) for most forest tree species. Altogether, they form a unique patrimony at European level, both quantitatively and qualitatively (genetic diversity): if conserved and managed properly, they constitute the reservoir for future genetic progress at European level. Forest genetic trials are another facet of this rich patrimony which allows species adaptation and genetic diversity study in an incomparable set of ecological conditions. Long-term conservation of these resources, archiving of related data for future investigations and globally access to experimental facilities appeared as key issues together with the standardisation of phenotyping and genotyping protocols.

TREES4FUTURE forestry project (I3 action, 2011-2016; 28 partners from 13 countries) moved several steps forward with an interdisciplinary approach and the creation of thematic networks, the provision of access to a diversified set of research infrastructures (28) and, the development of scientific tools of great interest firstly for the scientific community but also for forest stakeholders. Among these, let us mention: information systems on genetic and environmental resources; an open-source integrated platform for statistical and genetic data analysis (BreedR); consolidation of molecular tools for a European platform for fingerprinting and traceability of forest material; climate matching tool and spatial models for species site-matching; large scale tools to assess goods and services, sustainability, and mitigation and adaptation strategies to climate change; high-throughput phenotyping methodologies for tree adaptation traits and wood properties; catalogue of standardised protocols for field experimentation. Development of these tools has been initiated to directly support breeding and more largely forestry research but above all, they are the cornerstones for a future European Tree Breeding Centre.

This European Tree Breeding Centre which is envisioned is seen both as a repository platform, an incubator for innovative research in breeding, genetics and biotechnology of forest trees and as a stimulator for reinforced cooperation in breeding; it will also be a vitrine for forestry stakeholders with whom interaction is crucial. It will provide and maintain tools and develop initiatives for collection, conservation and enrichment of genetic resources and associated data; it will facilitate and organise access to these resources; it will encourage and support ambitious trans-national breeding programs for current major forest tree species but also for emergent ones; it will foster joined front-science research related to forest tree adaptation, genetics, breeding and biotechnologies. Training of researchers and interaction with stakeholders are also seen as key features of the missions for this European Tree Breeding Centre.

In a context of uncertainties but also of unpreceded challenges, the European Tree Breeding Centre will provide a stable, sustainable and incentive framework to researchers for a more reactive and efficient improvement of European forests resources. Benefits have been clearly recognised by stakeholders in a recent survey organised in the frame of this project (Country perspectives on tree breeding by Marzano et al.).

1.4 Potential impact

1.4.1 A European platform for research data on trees

To address the grand challenges, decision makers need an improved centralised genetic knowledge base, which sets clear demands for research. The centralized access point facilitates more effective access, sharing and use of data and information within and among different communities. This can without doubt lead to a more efficient exploitation of data resources and ultimately to easier transfer of advanced knowledge to society.

The Clearing House uses a self-updating architecture. The practical implication is that if changes are made to the data sources registered with the Clearinghouse, the Clearinghouse automatically becomes aware of these changes. This design means that the service will not lose value due to a lack of active maintenance.

End users can use the semantic search facilities of the Clearinghouse to discover forestry datasets. For that purpose, a graphical user interface (GUI) was developed that allows a user to query dataset on a combination of thematic (keyword search), spatial and temporal conditions. Figure 7 provides a screenshot of the GUI, displaying an example query and the associated search results.

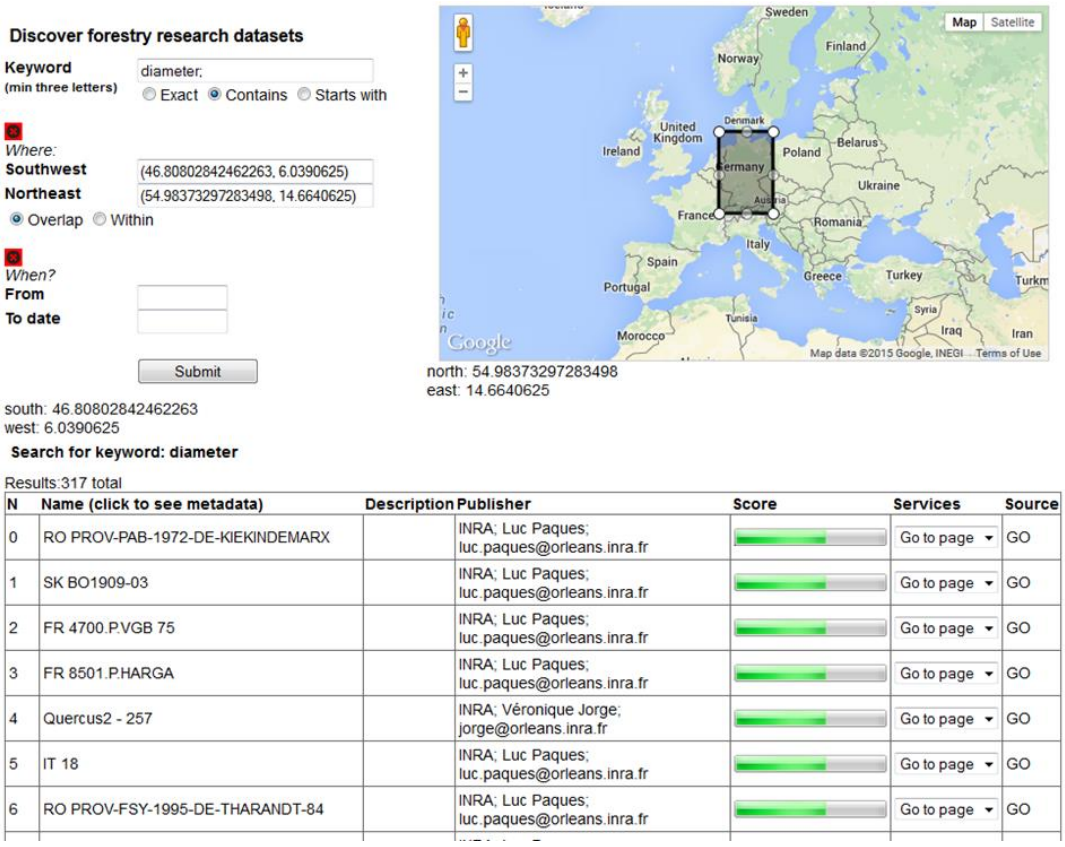

Figure 19. Trees4Future Clearinghouse semantic search interface

The T4F web portal also served as the primary source of information on T4F for project external people, including information for TNA application. According to D.4.6:

"The Trees4Future domain fees for www.trees4future.eu will be covered by EFI for the coming 2 years. At some point the website will be moved under the EFI web domain (e.g. www.efi.int/trees4future or trees4future.efi.int), with a redirect from the T4F domain name. This way people and search engines will have enough time to update their bookmarks and links to the migrated website/portal address. This will allow for the website to remain online at no fixed domain cost for 5 years after project end, but it will not be updated. A note will be placed on the main page stating that the project ran from November 2011 until April 2016 and that the current site serves as an archive of the activities and results of the project."

1.4.2 Standardisation of protocols for field assessment of traits: need for a common language

Socio-economic impact

Forest tree breeding has become an integral part of bio-economics by increasing and improving the quality of wood-based raw materials through selection, field testing and controlled crossings of different tree species. Breeding programs started in the mid-twentieth century and since then several European breeding institutions developed their own methodologies for assessment of various traits linked to the development of genetically improved forest material.

However, these different methodologies impeded the mutual comparison of results between institutions as well as between test series and single trials.

On the other hand, international collaboration becomes increasingly important as forests and trees are dealing with an ever changing climate. The challenge of environmental instability urges for new answers that can only be given through international collaboration networks. International common garden experiments established during the last century have to be reviewed in order to identify gaps in our knowledge and, thus, to provide a solid ground for future breeding programs in the frame of climate change. Common protocols for assessment of traits in these genetic trials as well as reference genotypes are essential to take up this challenge.

Exploitation of results

Several protocols have already been used at an international level.

In 2009, a pan-European provenance trial (*common garden experiment*) of wild cherry (Prunus avium) was initiated. The project was coordinated by the Flemish *Research Institute for Nature and Forest* (INBO), involved 16 research institutes active in forest tree breeding and aimed at the comparison of 23 European provenances defined at the seed source, seed stand and orchard level. Based on the outcome of the Trees4Future project, common protocols were adapted for stem straightness, branching habit, flushing, bud set and resistance/tolerance/susceptibility towards cherry leaf spot disease (*Brumeriella jaapii*).

Poplar protocols and reference genotypes were already adopted in an international field trial established in 2013 with several poplar genotypes and aiming at the selection of superior clones for the production of short rotation biomass (Coordinator: Randolf Schirmer, ASP, Germany). All 12 European partners agreed on some common protocols to be used for assessment of disease resistance and growth characteristics as described within the Trees4Future project. Unlike previous trials, the data collected by all partners will not need any adjustment and, thus, statistical analyses are quite straightforward. Several poplar common protocols have also shown their practical value during the FP5 project *Popyomics*.

1.4.3 High-throughput phenotyping: an urgent need for forestry research (Coordination: L.E.Pâques, INRA)

Development of high-throughput phenotyping methodologies and tools for a diversified range of traits will greatly improve characterization of tree phenotypes and wood samples. This is particularly true in two different contexts:

a) In tree breeding and genetic studies

Tree breeding aims at genetically improving properties of species through recurrent selection cycles, combining crossing, testing and selection. Selection is most usually multi-traits, including adaptive traits to biotic and abiotic factors, growth, stem architecture and branching and, more frequently wood properties. Testing in both genetic and breeding studies is achieved in diverse genetic trials types (provenance, progeny, clone) where hundreds of genotypes are evaluated across several sites over a few decades of years. Clearly this means that thousands of trees have to be repeatedly field measured during the experimentation. Therefore, non-destructive, fast, low cost and reliable assessment methodologies are highly wished to reduce cost, save time, and improve evaluation and selection efficiency. That is true in classical breeding schemes but even more in genomic selection where evaluation of large numbers of trees for many traits is necessary. Excepted for growth traits, several traditional methods, currently used to assess other traits, are not suited to the required level of throughput; some are too much subjective (*e.g*. phenology); some are destructive (*e.g*. for most wood traits assessment) or too complex to implement.

Automation of phenology monitoring through cameras, LED captors and dendrometers on one side; NIRS models for assessment of complex wood traits including adaptive traits such as hydraulic properties; multi-samples/ multi-traits potential for assessment of wood traits as shown by 2D NIR scanner on the other side, will greatly contribute to alleviate and enhance phenotyping of key parameters. For breeders, additional criteria for approving such new methodologies concern the level of heritability (genetic control) of the proxy used, its variability and its relationship with other traits. If these parameters are altered, they might change levels of expected gains and thus invalidate the choice of this new proxy. Nevertheless, a tolerance to reduced values of these parameters is accepted when one considers that genetic gains over a breeding cycle should be calculated by unit of time (or unit of cost). When one knows that traditional methodologies for assessing some traits can last several days, and even weeks (*e.g*. decay resistance to fungi), benefits of new proxies become evident. That is what we have demonstrated through several examples in a presentation at the final project conference in Brussels (Karkkainen and Pâques, 2016).

b) In wood industry

Characterization of wood products, right in the forest on standing trees or at the factory before and along the processing, allows a better choice of trees and their affectation to a given process. It allows then a better sorting out of products and their commercial valuation. This is valid for any anatomical, physical, mechanical, chemical or biological (durability) traits accordingly to industry process. NIR spectrometry models developed in the project for several properties (*e.g*. lignin, extractive content, MOE, tension wood, decay resistance) allow a fast and cheap determination of wood properties. Analysis can be done in a few minutes/sample on wood powder but to further accelerate the process, development was oriented towards spectral acquisition right away from solid wood, including small non-destructive samples such as increment cores. The possibility to predict several complementary wood properties from a single operation of spectra acquisition, to access to their radial variation

within the trunk, and eventually to analyse at once several tens of samples as the possibility was illustrated in this project thanks to a 2D-Scanner should directly benefit to industry. Expertise in NIR spectrometry applied to wood products is well-developed in the consortium and further cooperation with industry is expected either to implement existing solutions or to develop jointly new models for properties of interest. That is one of the objectives to develop further a pan-European NIRS platform for wood traits.

1.4.4 Towards a collaborative development of tools for statistical analysis of forest genetic resources

breedR development and release have three main impacts in our community. The first of them has been fostering the exchange of expertise on statistical issues in forest genetics across the T4F consortium and beyond it at international level, through dissemination tools, training efforts and via beta-testing networks. This exchange is still ongoing after the end of T4F, mainly through mailing lists involving users and breedR forge. We can say now that partners have a clearer idea of who's who and who does what in terms of statistical analyses of forest trials.

The second impact starts to be evident in the emerging community of users. It concerns the fact that the breedR community is embracing a set of novel statistical approaches with the potential to improve our analyses. In a few words, these novel approaches in breedR improve the assessment of causal variance components and individual evaluation in forest trials beyond what is attainable from classical approaches. In order to attain this, we have implemented methodologies that account more precisely for environmental factors that affect tree performance, like competition and hidden spatial patterns in trials, by integrating underlying relatedness information from molecular markers, and by accounting for interaction terms of tested varieties over macro-environmental clines. All these developments are in the framework of a well proven and flexible methodology of mixed models.

A third impact is still to come, and concerns the fact that new literature and documentation using breedR are underway among users. This process is expected to boost previous forecited effects.

1.4.5 Modelling of spatial deployment of species/provenances and of forest resources

By placing these models in sequence, the effect of dynamics outside the scope of one model can be taken into account when running another, while at the same time avoiding tedious and specialist model adaptation and calibration. The potential impact lies in the fact that genetic adaptation as know from provenance results and lab results, can now be modelled at large scale. Population adaptation can be assessed now.

The coupling of ForGEM and ORCHIDEE was a new coupling, i.e. the models have not been coupled before. Even though both models use the Farquhar representation of photosynthesis and a similar approach for stomatal conductance. The differences in implementation made that the sensitivities between the models differ. ForGEM was much more sensitive to changes in the selected parameters than ORCHIDEE. Hence, even a minor adaptive response, has rather strong effect in ForGEM, but hardly any in ORCHIDEE. Our overall conclusion is that for a sound transfer of the effects of adaptation from one process model to the other, the process description within these models should fully coincide, with respect to the selected parameters. The current result is that the coupling of ForGEM and ORCHIDEE currently does not provide improved insights on how genetic processes at the tree population level, affect vegetation development as simulated by ORCHIDEE.

Thus the consequences on on forest resource development and forest wood chains can currently not assessed by the coupled version of these models.

The coupling of EFISCEN to ORCHIDEE was new as well. We implemented the coupling by scaling increment rates according to EFISCEN empirical growth functions to changes in growth, as estimated ORCHIDEE. This approach itself is, however, not new and has been implemented previously by linking EFISCEN to other biogeochemical models (e.g. Schelhaas et al. 2003, Eggers et al. 2008; Seidl et al. 2014; Schelhaas et al. 2015). We used the relative NPP, rather than absolute NPP as a proxy for the change in growth rate, and applied this relative NPP as a factor on the regionally calibrated growth curves in EFISCEN. The approach for informing EFISCEN with expected changes in growth rate from bio-geochemical models, such as ORCHIDEE, is flexible but simple; there are strong assumptions e.g., on the correlation of growth rate and calculated change in NPP and on the matching of species used in the different models . Acknowledging this, the use of the change in NPP from ORCHIDEE, or any other climate-sensitive process based model, as information on spatial or temporal trends in the growth-rate of different tree species is useful when assessing regional differences in climate change effects on the forest sector. Furthermore, other processes that are

The models ToSIA to EFISCEN have been coupled previously in the FP6 EFORWOOD project by inserting EFISCEN results into the ToSIA database to initialise the FWCs with information on forest resources and harvest. To elaborate the coupling of the two models we implemented a tool that can connect to the EFISCEN database and extract relevant information to initialise FWCs. The coupling has been designed such that ToSIA can use any information stored in the EFISCEN output database, which is not limited to area, growing stock and harvest data. Furthermore, the tool can access EFISCEN outputs from previously (published) model applications. This feature gives freedom to a user as there is no need to run EFISCEN and ToSIA in parallel. It is important, however, that metadata on EFISCEN results is available, documenting in detail the assumptions on the scenarios for which EFISCEN outputs are available.

The climate matching tool produced by Trees4Future (Figure 20) and accessible on-line (http://193.185.149.20/t4f/cmt/), for a user selected climate projection at a user selected location, provides a spatial estimate of the best match to that future projection in the current climate. The tool therefore shows the climate analog space of future climate projections for a given location. We have additionally added uncertainty estimates of climatic moisture deficit, and the soil available water capacity to help users assess the vulnerability of species of tree or provenance, with different tolerances to water deficit, to projected climate conditions.

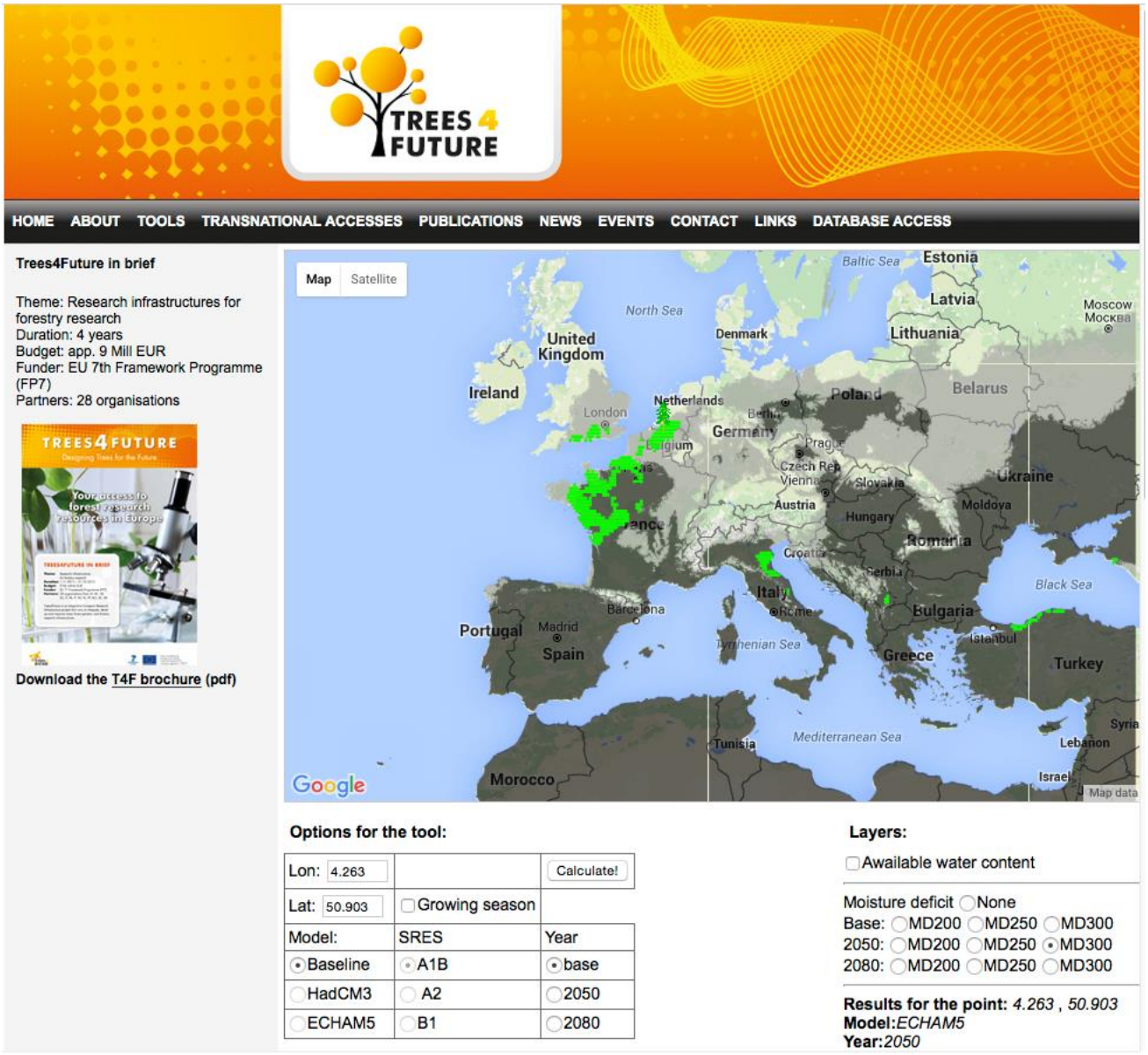

Figure 20. Trees4Future Climate Matching Tool output (http://193.185.149.20/t4f/cmt/)

1.4.6 Networking infrastructures and experts for forest research

The results of this Work Package are likely to affect trade in forest tree seeds and plants (or Forest Reproductive Material, FRM). With available genetic methods, greater consumer confidence in the quality of the product (FRM) can be achieved. The laboratories collaborating in this project have already established a reputation for themselves in their own countries. The research institutes and universities that have the necessary infrastructure in place can obtain the know-how from the project partners involved, by utilizing the descriptions of progress on genetic markers (as these are readily interpretable and usable for persons 'skilled in the art'). The way of interaction with stakeholders, as realized in this project, can serve as a model for other countries and regions in Europe: pilot studies, followed by coverage in forestry media, and direct interaction with stakeholders at local meetings.

Once greater consumer confidence is established, it can lead to a pricing differentiation on the market, with higher-value FRM products priced at a level that is attractive enough for producers. A key problem in FRM trade today is that often, the higher value of genetically tested or selected material is not honoured by the markets, as this value is not readily apparent visually from the seed or plant sold. Genetic markers can change the game in that they are able to certify this 'invisible' quality. With the confidence in the product, consumers (forest owners) can now plant the forests that they expect to better withstand future climate conditions, provide stable ecosystem services, and produce valuable timber in reasonable time, depending on the targets of forestry at the particular forest site. With the assurance on genetic quality, it is easier for them to accept higher prices, which again should stimulate more production of such better FRM.

This in turn should lead to better forests, a good that is not just important for the owner, but for European society in general. While this impact is very long-term, and the 'mass momentum' of the partners united in this project may not yet suffice to create this switch in the market, we are confident that by and by, all stakeholders will become much better aware of the possibilities of genetic laboratory analyses for quality checks on FRM, and that this increased awareness will bring about the described changes.

1.4.7 Improved access to forestry research infrastructures

Looking at the final outcome of the transnational access program, the relatively high number of approved projects out of the total submissions (only 1 project was completely rejected by both reviewers!) is probably due to the relatively low amount of the daily allowances granted to users. Only motivated researchers applied for the transnational access, being ready to face expenses barely covered/reimbursed by the program. Another factor that certainly improved the scientific quality of the project was the tight collaboration between applicant and Site Manager both in the preparation of the full applications and in the post-reviewing stage. Site Managers often "fell in love" with the projects submitted to get access to their site and they helped the applicant to write and sometimes re-think their goals, methods, aims. During the 2nd Annual Meeting in Tulln in November 2014, as well as at the Final Meeting in Brussels in April 2016, we hosted some "success stories" where users (2 in Tulln and 2 in Brussels) were invited to describe their project. In these cases, and in most other cases, the tight collaboration developed with Site Manager opened new roads and was very useful both for the applicants and for scientists at the hosting lab. It is certainly difficult to measure the outcome of these interactions and collaborations: many accesses gave start to new collaborations in other projects, too, that's what Site Managers confirmed during the Final Meeting. Publications made thanks to the transnational access program were a total of 56, mostly articles on scientific journals, but we assume that many will follow, as publications usually collect all results of the researcher's project, and not only the outcome of the single visit within our program. In fact, many publications dated 2015 or 2016 were made by T4F users who were granted support in 2012 or 2013.

1.4.8 Strategic Innovation and Research Agenda and European Tree Breeding Centre

The Ministers for Agriculture and Forestry of the EU member states proposed a budget of more than 5 billion Euros for the implementation of the Forest Strategy (of May 2014) until 2020 (including Research, Technology Development and Innovation – RTDI). The forestry research community should set the goal of at least 500 million Euros of that budget to be invested into RTDI. The **Strategic Innovation and Research Agenda** is therefore published in the right time and can help

advocating where the EU should direct strategic RTDI and infrastructure support for the highest impact and for the best result. The SIRA is a strong tool as its development was contributed to by a highly significant cross-cut of leading scientists and the findings of the consultation were implicitly supported by the outcome of broad-based stakeholder analysis. 300 hardcopies were distributed and an online version of the SIRA policy brief remains available on-line.

The development of the concept of a **European Tree Breeding Centre** is highly significant as such centre will form a flagship demonstration of decades of investment into tree breeding and genetics research and related infrastructure development. It would be a major leap for the coordination of efforts that are needed in readying European forests to provide a resilient and sustainable supply of wood raw material to supply the Green Economy while providing as well a multitude of ecosystem services, while climate change impacts are increasingly putting their detrimental mark on forest development and wide-scale implementation of adaptation measures are urgently needed.

The **models and tools** that have been upscaled and refined in the course of the Trees4Future project, each of them, represent important steps forward on the development of more integrated and intercommunicating tools. The publication of several of the key tools as open-source in open-source platforms increases the way forward with collaborative and reproducible research.

The **Clearinghouse** in its self-maintaining and updating design is an important step forward in the development of forestry and genetics clearinghouses. Typically the development of databases and clearinghouses is funded. However the maintenance of such is much harder to get funding for. Hence the self-maintaining architecture makes for an enduring impact of this product.

1.5 Address of the project public website, if applicable as well as relevant contact details.

http://www.treees4future.eu