Young scientist contract (PhD) offered by INRA within the doctoral school RP2E (Université de Lorraine)
Call for applications - April 2015

Inra and the Doctoral School RP2E offer during 2015 one "Contrat Jeune Scientifique" (Young Scientist Contract) for 3 years (duration for completion of a PhD). Research will take place at Nancy (France). Candidates may choose among the five topics offered this year:

Topic 1

Calcium, magnesium and potassium sources in the soil and their bioavailability in forest ecosystems: identification and quantification of bioavailability processes in *ex situ* conditions at a very fine scale.

Topic 2

Upscaling morphological, physiological and molecular determinisms of transpiration from the leaf level to water use at the whole plant level in poplar trees.

Topic 3

How are ectomycorrhizal fungi modulating plant hormonal signaling pathways, in particular jasmonic acid?

Topic 4

Intra-annual dynamics of wood formation and carbon sequestration in conifer and deciduous temperate forests

Topic 5

Understanding detoxification systems developed by wood-decaying fungi and potential application to evaluate the natural durability of wood

The topics and contact persons for specific information are provided in the documents attached to this announcement.

Applications

Applicants to this contract should send the application files including:
- A detailed CV with all details about obtained degrees, fulfilled training and results,
- A motivation letter indicating the selected topic and the plans of the candidate for his/her future career,
- A recommendation letter provided by a professor or a researcher who supervised the candidate during his/her training.

The candidates should hold a Master's degree in life sciences with an excellent grade list. The ideal candidate should demonstrate an interest in transdisciplinary research. He is expected to be creative and open-minded and to have the ability to establish and maintain good interpersonal relationships. Knowledge of French language is not a prerequisite. It can be learnt during the PhD. The candidates should have a good level of spoken and written English, and the thesis may be written in English.

The research will take place at Université de Lorraine, Science and Technology Faculty (10 min from city center by tram), or at the Inra campus, Champenoux (30 min by bus). The research groups are all members of the "Laboratoire d'Excellence" ARBRE (http://mycor.nancy.inra.fr/ARBRE/). In these groups you will benefit from the support of advanced technical platforms devoted to genomics, stable isotopes, electronic and confocal microscopy, wood science, etc… You will be member of a large-scale research community of about 350 persons working in forest and wood sciences (see http://www.nancy.inra.fr/).

Nancy is a medium-sized city (350000 inhabitants including suburbs) located in North-Eastern France. The city is very attractive in terms of gastronomy, cultural activities, architectural (ranging from the UNESCO classified Place Stanislas (18th century) to the so-called 'Ecole de Nancy' style),....The countryside is very peaceful with lakes and many forests, close proximity to the Vosges mountains (for skiing, trekking, mountain biking...), to Belgium, Luxembourg and Germany. There is very easy access to Paris (1h30 to Paris with the high speed TGV train) and to other destinations in France and the continent (30 min from National Airport Nancy-Metz, 1h15 from Luxembourg International Airport).

Applications files should be sent to the administration of the Doctoral School (christine.fivet@univ-lorraine.fr) with a copy to the president of INRA – Nancy (celine.ranger@nancy.inra.fr and camille.huysentruyt@nancy.inra.fr) before Friday May 17th, 2015.

A selection committee will examine all applications and will select the candidates for an audition, based on skills and adequation to the selected topic. The final selection will follow the audition of the candidates (Beginning of June 2015). Each audition will be based on a 15 min presentation followed by 20 min questions. The audition can be organised with a video conferencing system.

For more information, please contact: celine.ranger@nancy.inra.fr or the scientist responsible for each topic.

RP2E website : http://www.rp2e.univ-lorraine.fr
INRA website: http://www.nancy.inra.fr

Research topic 1

Calcium, magnesium and potassium sources in the soil and their bioavailability in forest ecosystems: identification and quantification of bioavailability processes in *ex situ* conditions at a very fine scale.

Research Unit:

UR-1138 Biogéochimie des Ecosystèmes Forestiers (BEF) (Centre Inra de Nancy-Lorraine)

Supervisors of the PhD thesis:

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General aims and state of the art:

In recent years, decreasing atmospheric deposition rates of Mg and Ca have been observed worldwide (e.g. Hedin and Likens, 1996) while N deposition has remained high, and potential negative effects on forest ecosystems have been reported (e.g van der Heijden *et al.*, 2011; Jonard *et al.*, 2012). On the other hand, the increasing wood demand and the intensification of silvicultural practices (thinning and harvest frequency, biomass export, etc.) may significantly impact soil fertility in forest ecosystems where the nutrient level is low (Thiffault *et al.*, 2011). The sustainability of forest ecosystems on base-poor soils is uncertain in such a context and is a growing concern in the international forest community: Jonard *et al.* (2014) reported a significant decrease in beech leaf concentration (Ca, Mg, P) over Europe; in Sweden, a national research program (QWARTS) is focused on quantifying the long-term supply of calcium, magnesium and potassium to forest ecosystems; in the Northeastern USA, reductions in exchangeable soil Ca and Mg have been associated with declines of sugar maple (Bailey *et al.*, 2004).

When the pressure endured by low fertility forest ecosystems is too intense, nutrient losses and ecosystem function losses (economic, ecological and social) occur. Restoration operations such as liming are a cure to forest ecosystem degradation but are very expensive. Understanding forest soil fertility and nutrient cycling is therefore essential in order to help forest managers and policy makers manage forest soil fertility in a sustainable manner and prevent cases of forest decline. It is however not yet clear how trees cope with very low nutrient resources. In spite of many detailed studies, input, output and internal fluxes of nutrients in these forest ecosystems remain very difficult to precisely measure and quantify. Numerous studies focused on forest soil fertility have reported discrepancies between the different approaches (input-output budgets, soil pool measurements, tree nutrition status, etc.). These discrepancies highlight the limits of our knowledge and of current methods used to study nutrient cycling and the interactions between the soil and the tree.

An *in situ* multi-isotopic ($^{2}H_2O$, ^{26}Mg, ^{44}Ca and $^{15}NO_3$) tracing experiment was carried out in 2010 in a 35-year-old beech stand at the Breuil-Chenue SOERE experimental site in order to better understand nutrient fluxes and cycling. This experiment showed that trees access a source of Ca that is currently not identified. This Ca source may be foliar absorption and/or Ca uptake or diffusion from deep sources but the main hypothesis is that current methods underestimate calcium pools which are truly bioavailable (exchangeable pool, primary and secondary mineral pool, organic matter, organo-mineral phase). These unaccounted sources may also, depending on the ecosystem, play an important role in Mg and K tree nutrition. However the *in situ* multi-isotopic tracing experiment does not enable to answer this new research question because too many factors vary simultaneously. Studying processes at a much finer temporal and spatial scale is necessary to understand soil fertility and nutrient cycling at the ecosystem scale.

Specific research topic:

This PhD project is focused on the identification and quantification of the sources of calcium, magnesium and potassium in forest soils and the bioavailability of these sources. The different soil pools of Ca, Mg and K are currently defined by the different methods of soil extraction: *e.g.* exchangeable pool measured by cationic exchange extractions, secondary mineral pools by TAMM extractions (oxalic acid), non-exchangeable interfoliar K etc. However, the bioavailable fraction of the different pools cannot be deduced from conventional extractions alone. We hypothesize that bioavailable pools of Ca, Mg and K in the soil are underestimated by conventional approaches. Conventional and multi-isotopic tracing approaches will be combined to study the interactions between the different soil sources and the tree in *ex situ* conditions at a fine scale. The objectives of this project are twofold:

1) Interactions between the different soil reservoirs of calcium, magnesium and potassium. In this first part of the PhD project the hypothesis that soil pools other than the exchangeable pool are in equilibrium with the soil solution, and therefore potentially available for plant uptake, will be tested. Multi-isotopic tracing using stable isotopes will be combined with conventional approaches in order to identify the labile (isotopic exchange/diffusion) pools of Ca, Mg and K and characterize the interactions, exchanges and diffusion kinetics between the different pools. This novel approach will be developed and applied on well characterized soils from the different high resolution experimental sites monitored by BEF, before being applied on a wider range of soil types from national and international soil data bases (RENECOFOR, ICP Forests, etc.) in order to identify the main factors influencing Ca, Mg and K bioavailability.

2) Interactions between the different soil reservoirs and the plant. In the second part of this project, the hypothesis that the potential bioavailable pools (labile pool) of Ca, Mg and K in the soil (identified and characterized during the first part) are available for plant uptake will be tested and the interactions between the plant and the different labile pools will be characterized. The project proposes to use plant-soil model systems, in which the soil pools will be selectively isotopically labeled, to quantify Ca, Mg and K fluxes between the soil pools and the plant and study the effect of plants on the fluxes between the different soil pools (rhizosphere effect).

Novelty and relevance to the research project of the team:

The results of this PhD project will enable to gain considerable insight into exchange and diffusion processes between the liquid and solid phase of the soil and better understand the processes involved along the path which leads nutrients from the soil solid phase to the root of the plant. The protocols and approaches developed and applied during this project will be transposable to other systems (forest ecosystems, agricultural systems, orchards, etc.) and to other research questions (e.g. root specificity for nutrient uptake, etc.). The results will also enable to develop biogeochemical models which are used to predict forest ecosystem sustainability. The experimental data set produced during this PhD project will be a corner stone to a future collaborative project with UCL in Belgium (Mathieu Jonard) to model nutrient transport from the soil to the root.

Potential impact for the scientific discipline and the society:

The results of this PhD project will enable to gain considerable insight into exchange and diffusion processes between the liquid and solid phase of the soil and better understand the processes involved along the path which leads nutrients from the soil solid phase to the root of the plant. The protocols and approaches developed and applied during this project will be transposable to other systems (forest ecosystems, agricultural systems, orchards, etc.) and to other research questions (*e.g.* root specificity for nutrient uptake, etc.). The results will also enable to develop biogeochemical models which are used to predict forest ecosystem sustainability. The experimental data set produced during this PhD project will be a corner stone to a future collaborative project with UCL in Belgium (Mathieu Jonard) to model nutrient transport from the soil to the root.

Currently, biogeochemical models generally consider the soil as a nutrient reservoir. The processes and interactions between the different compartments of the soil are most often not implemented. This project will enable to estimate bioavailable pools with more precision in the soil which will, in turn, help predict more accurately the risks of forest ecosystem degradation.

Available equipment / experimental support / associated research projects:

The BEF unit has developed over the past decades a strong scientific and technic (analytical methods) expertise in the use of stable isotope tracing (^{15}N, ^{13}C, $^{87}Sr/^{86}Sr$, ^{44}Ca, ^{26}Mg, ^{11}B) in both natural abundance and labeling approaches to study nutrient cycling from the process to flux scale. The BEF unit is equipped with a clean lab to prepare samples for isotope analysis and with an ICP-MS instrument for Mg, Ca and K isotope ratio analysis in isotopically enriched conditions. The BEF unit is also equipped and has strong expertise in the use of conventional approaches (different soil extractions, elemental analysis, mineralogy, etc.). The experiments planned in the framework of this PhD project may be rapidly carried out since the necessary amount of isotopically enriched material (^{26}Mg, ^{44}Ca and ^{41}K) have already been acquired. Finally, the INRA center is equipped with greenhouses which will enable to set up the plant-soil model systems in controlled environment conditions (temperature, soil moisture, etc.).

Skills that the doctoral fellow will gain during the contract:

The doctoral fellow will gain skills in soil science (characterization and extraction of different soil nutrient pools) and stable isotope tracing (experimental design and isotope ratio analysis). The fellow will also gain experience in the field of nutrient bioavailability.

By conducting this research project, the fellow will also develop many working skills such as project planning, interacting and animating a research team and managing large data sets.

Five publications of the research group on the topic:

van der Heijden, G., Dambrine, E., Pollier, B., Zeller, B., Ranger, J., Legout, A., 2015. Mg and Ca uptake by roots in relation to depth and allocation to aboveground tissues: results from an isotopic labeling study in a beech forest on base-poor soil. Biogeochemistry 122, 375-393.

van der Heijden, G., Legout, A., Pollier, B., Ranger, J., Dambrine, E., 2014. The dynamics of calcium and magnesium inputs by throughfall in a forest ecosystem on base poor soil are very slow and conservative: evidence from an isotopic tracing experiment (26Mg and 44Ca). Biogeochemistry 118, 413-442.

Bolou-Bi, E.B., Vigier, N., Poszwa, A., Boudot, J.P., Dambrine, E., 2012. Effects of biogeochemical processes on magnesium isotope variations in a forested catchment in the Vosges Mountains (France). Geochim Cosmochim 87, 341-355.

Cobert, F., Schmitt, A.-D., Calvaruso, C., Turpault, M.-P., Lemarchand, D., Collignon, C., Chabaux, F., Stille, P., 2011. Biotic and abiotic experimental identification of bacterial influence on calcium isotopic signatures. Rapid communications in mass spectrometry : RCM 25, 2760-2768.

Research topic 2

Upscaling morphological, physiological and molecular determinisms of transpiration from the leaf level to water use at the whole plant level in poplar trees.

Research Unit: UMR-1137 Ecologie et Ecophysiologie Forestières (EEF) (Centre Inra de Nancy-Lorraine, Université de Lorraine)

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General aims and state of the art:

Ongoing climate change is predicted to result in a higher frequency of dry and hot summers (IPCC, 2007) even for temperate regions, comparable to those observed in 2003 and 2005. This increases the risk of water supply problems for tree plantations as well as for natural populations, with a risk of decrease in wood production, or even a reduction in land-area suitable to grow poplar. Therefore the optimization of water use for the production of biomass is an important research aim in poplar. Water use efficiency (WUE) is defined as the ratio between the amount of carbon accumulated in biomass and the amount of water transpired by a plant, for a given period of time. At the leaf level, instantaneous, intrinsic WUE is defined as the ratio between net CO_2 assimilation and stomatal conductance to water vapor (W_i), and can be measured directly using gas exchange equipment, but also estimated indirectly using the carbon isotopic composition of plant organic material ($\delta^{13}C$) (Farquhar & Richards, 1984). Whole plant WUE, called here transpiration efficiency (*TE*) is estimated by lysimetric methods to determine daily water loss and allometrics/destructive harvesting for biomass accumulation. *TE* at whole plant level is controlled by: (i) intrinsic WUE of each leaf (Condon *et al.* 2004); (ii) the vapour pressure deficit from leaf to air (VPD_l) which may increase directly transpiration but also decrease stomatal conductance (Lange *et al.* 1971; Monteith 1995) and (iii) scaling factors from leaf to whole plant, like relative carbon (Φ_c) and water (Φ_w) losses not associated with photosynthesis (Farquhar *et al.* 1989). Φ_c depends on the intensity of respiration in stems and roots, and on whole plant nocturnal respiration, while Φ_w depends on water losses by stems and fruits as well as on nocturnal transpiration. Further, the plasticity of intrinsic WUE among leaves from one plant (Le Roux *et al*., 2001) and their relative contribution to the whole plant carbon and water budget may impact *TE* at whole plant level.

Since 2003 research teams of INRA/University at Orleans and Nancy have evidenced a clonal diversity of WUE in the hybrid formula *Populus deltoides x nigra* (Euramerican poplars) and its possible relationship with productivity. The aim of this collaborative research was to identify the functional leaf traits involved in diversity among clones and the most striking result was the identification of a large diversity of $\delta^{13}C$ and W_i. The relative differences in WUE among clones were maintained in situations of drought obtained by stopping irrigation in a field experiment (Monclus *et al*., 2006). We have further shown, using a diachronic approach (assay of $\delta^{13}C$ in cellulose of trees and monitored over time), that genetic differences detected at a young age (5 years) are maintained during the ageing of the trees in plantations (Rasheed *et al*., 2011). For *Populus nigra*, similar differences of WUE (including whole plant and leaf level WUE, carbon isotope discrimination) were evidenced among young cuttings in a greenhouse (Rasheed *et al*., 2013). However, such comparisons of genotypes of different age, size or under different experimental conditions are not yet fully matched by comparable measurements on greenhouse grown one year old cuttings or planted trees in a common garden. Moreover, cutting edge results on transcriptomic and metabolomic responses of poplars to drought are usually obtained for greenhouse grown plants, and the transfer of knowledge to the whole tree level in a natural environment would be crucial for their interpretation.

We have over three PhD theses (Monclus, 2006; Fichot, 2010; Rasheed, 2012), increased our knowledge about the determinism of the variability of W_i among genotypes by assessing the related physiological and anatomical traits. Very close relationships were found between W_i and stomatal conductance and stomatal density; they are inverse to those found in pedunculate oak (Roussel *et al*. 2009; Vialet-Chabrand, 2013), which suggests the involvement of other traits such as stomatal size and speed of opening-closing of stomata. We found that, among *Populus nigra* genotypes, the differences of intrinsic water-use efficiency at leaf level where matched by similar differences in whole plant *TE*. This was true under two levels of *VPD* despite the fact that higher *VPD* induced some degree of stomatal closure. Nevertheless, mesophyll conductance may differ among genotypes, and in response to *VPD*, which may to some extent bias the upscaling process. Similarly, we cannot exclude genotype-induced effects on Φ_w and/or Φ_c. Recent research in our group (Vialet-Chabrand *et al.,* 2013) proposed a new model, which allows to assess the dynamic responses of stomata to environmental variations. Differences in these dynamic responses among genotypes may affect the daily-cumulated water use efficiency. **Therefore, a major challenge when upscaling to older and larger trees, will be to characterize (i) the dynamics of stomatal closure and opening and their contribution to whole-plant carbon and water budgets; (ii) the plasticity of stomatal density and anatomy within trees and (iii) the potential contribution of Φ_w and/or Φ_c which may gain importance when tree size increases.**

Specific research topic:

The PhD project will be structured around three questions :

- Are differences in stomatal behaviour among poplar genotypes changing between measurements in controlled and in field conditions? And related to which environmental variables (as light intensity and quality, VPD, leaf temperature and soil water content)?
- How does this influence water use efficiency at leaf and whole plant level?
- Which traits influencing stomatal functioning (at morphological and molecular levels) are involved in closure and opening processes (speed and amplitude of responses) ?

Hypotheses

- The mechanisms controlling the transpiration efficiency are mainly depending to those controlling leaf water use efficiency.
- The genotypes have different stomatal conductance regulations and thus show different plasticity in response to soil water conditions
- The modification of leaf anatomy (ontogeny of stomata) is a means of stomatal acclimation to a lower soil water content.
- Some functions, linked to the sensitivity and the speed of the response of stomata to various environmental stimuli are the same between all leaves and can be used to model whole tree responses.

Novelty and relevance to the research project of the team:

UMR EEF (Ecology and Ecophysiology of Forests) is a joint research group between INRA and University of Lorraine. EEF implements multi-scale research programs aiming at investigating short and long- term effects of environmental stresses on tree function and growth, on water and carbon cycle, on forest species distribution and its temporal changes. EEF focuses on various stresses: water shortage and excess, high temperatures, carbon dioxide and ozone concentration in the atmosphere. This research covers a broad range of experimental approaches: from gene expression to the mechanisms of root growth and of stomata functioning, to integrative biology at the tree scale, to forest ecosystems, to the region and the country. The proposed PhD project completely fits with these objectives. EEF is also deeply involved in transfer of knowledge to stakeholders and forest managers who address questions on the future tree species and on new management rules face to extreme events and climate change. Research activities are organized along three axes: 1) Physiology and Diversity of responses to constraints; 2) functional ecology of tree and ecosystems and 3) long-term dynamics of forest ecosystems. This project will also coherently complete the main project of the PhysioDiv team. To date, studies that address changes in scales are rare. In this context, this project is particularly original, because it will focus on mechanisms involved in water use efficiency at leaf scale and transpiration efficiency at tree scale. A better understanding of such stomatal processes should also allow to improve other models (Castanea, Orchidee, Maestra, FORGEM...) at larger scales on canopy functioning.

Potential impact for the scientific discipline and the society:

The question of global changes and their effects on forest ecosystems is currently at the heart of concerns, including those of managers and stakeholders. The demand for research and forecasting on these issues became very strong at the national but also international level. In the Strategic Framework 2010-2015's the Department EFPA, two challenges are put forward (i) assessing the environmental risks to better manage ecosystems and (ii) assessing and promoting the adaptation to global changes in forests, grasslands and aquatic environments with a priority of research 'study the adaptation of organisms and populations to global changes - development of indicators of adaptability'.

Drought is one of the most important abiotic stresses limiting the growth of trees (Aussenac, 2000) and will of increasing importance for forest plantations in many countries in the coming decades (Dvorak, 2012). With the global changes expected in the next few decades, areas affected by this constraint will be expanding. Trees will face periods of drought without having the ability to adapt over time through natural selection. To predict the consequences on the dynamics of forests and the productivity of plantations requires the understanding of the mechanisms of acclimation of trees to water deficit and the evaluation of intra and interspecific response. This thesis project is fully in agreement with these objectives and aims to understand the nature of the changes, in response to water stress, operating in the physiological, anatomical and molecular levels. We will pay particular attention to develop research: (i) to provide new knowledge on the physiological impacts of drought, (ii) to characterize the process of acclimation induced by this constraint (phenotypic plasticity), (iii) characterizing the intraspecific diversity of functional traits likely to be under a natural selection, (iv) bringing knowledge at tree level which can be integrated across the forest.

Available equipment / experimental support / associated research projects:

This study will be performed on poplar, a model tree in genomics studies since its sequencing in 2004 and the availability of many related tools (databases, chips, ...). Four genotypes of poplar (2 euramerican genotypes: Carpaccio et I214 and 2 black poplars 6J29 et N38) have been selected according to their tolerance to drought (maintaining standards of productivity and ability to close the stomata, results obtained from previous studies, such as the European project WATBIO and the ANR project POPSEC). Methods for the application of the water stress will vary depending on the experimental objectives, we will achieve experiments in semi-controlled conditions (INRA greenhouses, irrigation control robots: relationships between weight-soil moisture). These trees will be planted in the nursery at INRA Champenoux in 2014 and will be subject to a reduced water input by rain exclusion. We have tools for analyses with the technical platform PTEF (isotopy and microscopy, scanning electron microscope, laser microdissection), the plateau of genomics (transcriptome), as well as all leaf gas exchange and sap-flow measurement devices. The models (photosynthesis, Farquhar; dynamic conductance, Vialet-Chabrand *et al.* 2013) are controlled by supervisors.

Skills that the doctoral fellow will gain during the contract:

The framework is committed to train the student to build the thesis project and more broadly his professional project. It is essential for the doctoral student that he possesses at the end of his thesis an assertive scientific identity. This will allow him to apply in the best conditions to scientific positions after completing his doctorate. Basic cognitive skills that will be acquired are as follows: capacity of synthesis, to situate of the context of the thesis in its local, national and international environment, to adapt the project issues, as well as written and oral scientific communication. The technical skills acquired will be the following: physiology of photosynthesis and leaf transpiration, histology, micro-sampling, microscopy, molecular biology, transcriptome analysis, measurements of sap-flow, statistic, parameterization of ecophysiological models.

This PhD research will focus clearly on questions at leaf level and whole tree level (at different age and environmental conditions : greenhouses and field), not at ecosystem level. The first two years will be dedicated to the acquisition of data in greenhouses (controlled conditions) and in field; the third year will be dedicated to the integration of data and to their publication. We envisage a post doctoral training period after thesis abroad to validate results acquired on ecosystem scale, this is why we invite two researchers (Hendrik Davi and Koen Kramer), experts on modelling, to participate to the thesis committee.

Five publications of the research group on the topic:

Dumont J, Cohen D, Gérard J, Jolivet Y, Dizengremel P, **Le Thiec** D. 2014. Distinct responses to ozone of abaxial and adaxial stomata in three euramerican poplar genotypes. *Plant Cell & Environment*, 37 : 2064-2076.

Marguerit E, Bouffier L, Chancerel E, Costa P, Lagane F, Guehl JM, Plomion C, **Brendel** O. 2014. The genetics of water-use efficiency and its relation to growth in maritime pine. *Journal of Experimental Botany* 65: 4757-4768.

Vialet-Chabrand S, Dreyer E, **Brendel** O. 2013. Performance of a new dynamic model for predicting diurnal time courses of stomatal conductance at the leaf level. *Plant, Cell and Environment* 36, 1529–1546.

Douthe C, Dreyer E, **Brendel** O, Warren CR. 2012. Is mesophyll conductance to CO_2 in leaves of three Eucalyptus species sensitive to short-term changes of irradiance under ambient as well as low O-2. *Functional Plant Biology* 39 (5) : 435-448.

Rasheed F, Dreyer E, Richard B, Brignolas F, Montpied P, **Le Thiec** D. 2012. Water-use efficiency of six *Populus x euramericana* genotypes : differences in ^{13}C discrimination between atmosphere and leaf-matter match differences in transpiration efficiency. *Plant, Cell & Environment* 36 : 87-102.

References

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Dumont J, Cohen D, Gérard J, Jolivet Y, Dizengremel P, Le Thiec D. 2014. Distinct responses to ozone of abaxial and adaxial stomata in three euramerican poplar genotypes. *Plant Cell & Environment* doi: 10.1111/pce.12293.

Dvorak WS. 2012. Water use in plantations of eucalypts and pines: a discussion paper from a tree breeding perspective. *International Forestry Review* 14 : 110-119.

Farquhar GD, Richards RA. 1984. Isotopic composition of plant carbon correlates with water-use efficiency of wheat genotypes. *Australian Journal of Plant Physiology* 11 : 539-552.

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Le Roux X, Bariac T, Sinoquet H, Genty B, Piel C, Mariotti A, Girardin C, Richard P. 2001. Spatial distribution of leaf water-use efficiency and carbon isotope discrimination within an isolated tree crown. *Plant, Cell & Environment* 24 : 1021-1032.

Monclus R, Dreyer E, Villar M, Delmotte FM, Delay D, Petit JM, Barbaroux C, Le Thiec D, Bréchet C, Brignolas F. 2006. Impact of drought on productivity and water use efficiency in 29 genotypes of *Populus deltoides* × *Populus nigra. New Phytologist* 169 : 765-777.

Monteith JL. 1995. A reinterpretation of stomatal responses to humidity. *Plant, Cell & Environment* 18, 357-364.

Rasheed F, Dreyer E, Richard B, Brignolas F, Montpied P, Le Thiec D. 2013. Genotype differences in 13C discrimination between atmosphere and leaf matter match differences in transpiration efficiency at leaf and whole-plant levels in hybrid *Populus deltoides x nigra Plant, Cell & Environment* 36 : 87-102.

Rasheed F, Richard B, Le Thiec D, Montpied P, Paillassa E, Brignolas F, Dreyer E. 2011. Time course of delta^{13}C in poplar wood: genotype ranking remains stable over the life-cycle in plantations despite some differences between cellulose and bulk-wood. *Tree Physiology* 31 : 1183-1193.

Roussel M, Le Thiec D, Montpied P, Guehl JM, Brendel O. 2009. Diversity of water use efficiency in a *Quercus robur* family: contribution of related leaf traits. *Annals of Forest Science* 66 : 408-417.

Vialet-Chabrand S, Dreyer E, Brendel O. 2013 Performance of a new dynamic model for predicting diurnal time courses of stomatal conductance at the leaf level. *Plant. Cell Environ. 36:* 1529-1546.

Vialet-Chabrand, 2013, Modélisation des variations journalières de la conductance stomatique: apports d'une approche dynamique et conséquences sur l'efficience intrinsèque d'utilisation de l'eau chez le chêne. Thesis, University de Lorraine, 5 september 2013

Research topic 3

How are ectomycorrhizal fungi modulating plant hormonal signaling pathways (in particular jasmonic acid)?

Research Unit:

UMR INRA/UL 1136 Interactions Arbres-Microorganismes, F54280 Champenoux

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General aims and state of art:

There is increasing interest worldwide in tree plantations, because of their use in the economy (wood, paper, resin), ecology (carbon sequestration), and bioenergy (source of heat and conversion of cellulose in biofuel). In this context, every component impacting tree productivity is studied. In nature, soil-born fungi associate with roots of trees to form ectomycorrhiza (ECM). These mutualistic interactions contribute to better plant growth and health via improving mineral nutrition, strengthening plant defences and direct contribution to exclusion of competitive microbes. Despite their ecological importance, ectomycorrhizal symbiosis is still not well understood at the molecular level, partly because of the complexity of eukaryotic cells and their multicellularity. To establishment, fungal hyphae from the soil first grow towards host root cells and encompass short lateral roots to form the mantle. Mycelia then colonize the apoplastic space forming the Hartig net, the symbiotic interface where efficient nutrient exchanges (P, N and C) as well as molecular dialogue take place. The orchestration of these morphogenetic elementary processes depends on both control of hormonal balance (e.g., auxins) and symbiosis-related gene networks that promote cell divisions and rearrangements, as well as regulate tissue size and stem cell activity. Recent studies highlight the importance of fungal small-secreted proteins/peptides (SSP)-based communication (dialogue) between plants and their symbionts. In the case of *Populus* colonization by *L. bicolor*, those SSPs with induced expression in mycorrhizal root tips were named Mycorrhiza Induced Small Secreted Proteins (MiSSPs). The functional analysis of *L. bicolor* MiSSP7 showed that this 7kDa-peptide is necessary for the establishment of symbiosis and that it interacts with the host protein PtJAZ6, a negative regulator of JA signalling pathway in *Populus*. The association of MiSSP7 and PtJAZ6 prevents JA-induced degradation of PtJAZ6, thus blocking JA-responsive genes, among them genes involved in plant immunity or plant cell wall remodelling. These results shed new light on how beneficial and pathogenic microbes have evolutionarily diverged in the mechanisms by which they overcome plant defences.

All results further the concept that there is a complex interplay occurring between the plant hormonal balance and the symbiotic effectors (MiSSP) to form a mutualistic relationship. It is likely that first ECM fungi manipulate the auxin gradient within the root, such that more lateral roots are produced and thus providing more surface area to colonize. Second, fungal effectors, such as MiSSP7, are likely produced to counteract plant defense responses (e.g. JA, ET) and create localized weakening of the plant immune system, which would then allow for the beginning of hyphal penetration into the root apoplastic space. A third wave of effectors, such as mycobiont encoded cell wall-active enzymes, then modify cell:cell attachments and plant cell wall rigidity, allowing further hyphal penetration within the root tissues. Once within the apoplastic space, and upon establishment of nutrient transfer, the hyphae must continually protect themselves from detection by the plant immune system, likely using masking proteins (e.g., hydrophobins) and decoys (e.g., MiSSPs) as diversionary tactics.

Consequently, MiSSPs are clearly at the heart of the molecular dialogue operating to promote symbiosis. Thus elucidating the role(s) of these MiSSPs is required and would help to better understand tree physiology from hormone-mediated signalling to C partitioning and bi-directional nutrient exchanges.

Specific research topic:

As an adaptation of trees to survive in nutrient –limiting conditions, ECM mutualistic interactions are crucial for tree growth and productivity. Over the last five years, we have focused our research on the molecular dialogue operating between the ECM fungus and root cells. Specifically, we focused our interest on peptides-based signalling namely Mycorrhiza-induced Small-Secreted Proteins (MiSSPs) of *L. bicolor*. These MiSSPs resemble effectors of pathogenic fungi, nematodes, and bacteria as they promote fungal colonization of the plant tissues, by down-regulating plant defence responses and controlling plant hormone balance. As hormonal balance is also important for tree physiology, it is crucial to better understand the role (s) of MiSSP on hormonal balance. It will constitute the main framewok of the PhD thesis.

Specifically, we propose to (i) dissect at the biochemical level how the MiSSP7-JAZ6 complex interacts with/modify the host targets to promote symbiosis establishment and (ii) to identify amongst the >50 L. bicolor MiSSPs those (if any) interacting with hormone receptors controlling plant defence reactions (salicylic acid, SA) or/andmycorrhizal root development (IAA, ET, gibberellins) and dissect their role at the biochemical level mediated signalling such as Salicylic Acid (SA) and Giberrelic Acid (GA).

Novelty and relevance to the research project of the team:

The significance of our study on how MiSSP7 is impacting jasmonic acid mediated signalling in Populus roots was highlighted by research highlight "ECM fungi and all that JAZs'" in Nature Reviews Microbiology and in Faculty of 1000. Characterizing interactions between plants and microbes is critical to achieving long-term ecosystem productivity and a full accounting of the carbon cycle in belowground, as well as aboveground systems. Subsequent reviews reflect an emerging understanding of molecular themes that recur across plant-microbe interactions ranging from pathogenic to beneficial. One of the most complex challenges faced by plant-associated microbes is to modulate the host's physiology in order to mitigate the defence response, gain nutrition and reproduce. Advances in genome-based studies on plant-associated microorganisms have transformed our understanding of many plant-associated fungi, in particular pathogenic ones. These studies have provided novel information about how filamentous microbial pathogens have adapted to particular hosts, changes in host range and the emergence of new pathogen species. Similar to pathogens in their way of host colonization while also similar to saprotrophs in their ability to mine forest soils for nutrients, mutualistic ECM fungi occupy an interesting niche between the two lifestyles. Recent studies emphasize the blurred lines between biotrophic pathogenic fungi and mutualistic ones. In particular, they are all dealing with hormone-signalling pathways, whatever their contrasted way of life is. However, whether biotrophic bacterial and fungal plant pathogens stimulate jasmonic acid signalling to promote colonization, the ECM fungus *L. bicolor* by contrast establishes its mutualistic interaction with plant roots by blocking this signalling pathway. This similarity in effector's target but the difference in the way of action highlight the need to better characterize and understand the roles of effector proteins and the targeted hormone signalling pathways used to facilitate mutualism.

This is clearly a challenging project that will require an efficient teamwork. However, demonstrating that receptors for major hormones involved in symbiosis development are targeted by a diverse set of fungal MiSSPs would revolutionize the current models of symbiosis development and plant-microbe interactions.

Potential impact for the scientific discipline and the society:

On short term the research included in this project will lead to novel insights into hormone signalling during ECM development and its control by *L. bicolor*. We expect to identify key factors for ECM development and functioning, as marker genes of well-functioning ECM. Results will be communicated to the scientific community by presentations on international and local conferences and by publication in high-impact journals. The precision and tuning of the mutualistic genetic blueprint is its biggest success, as demonstrated by nearly all land plants being colonized by mycorrhizal fungi, but it is also its biggest weakness. Interruption of any of the finely tuned symbiotic signalling pathways is likely to greatly change the nature of the interaction between the cooperating organisms, if not completely abolish the mutualism. This is why excessive 'mining' type management of vast stands of boreal forests and the forecasted effect of climate change are such a threat to these ecological systems. By using candidate markers identified within this project, we will have the tools necessary to evaluate ECM functioning *in situ*. By studying these systems, and their adaptive plasticity under different parameters, we will know better how ECM fungi will fare under new climactic conditions, and we will know how to better manage the microbiome of forests – a grand challenge in biological and environmental research.

Available equipment / experimental support / associated research projects:

To assure a proper performance of the described experiments facilities for standard microbiological work (e.g. laminar air flow and incubators) and in vitro plant culturing (e.g. phytotron) are required. Additionally, equipment for molecular biological work is needed. Computer clusters and software for transcriptome analyses and network modelling are necessary. All these facilities, materials and know-how are available in the host unit. Financial resources to cover costs of sequencing, consumables, reagents, etc. are required: 150,000 € is available through the Lab of Excellence ARBRE project SYMWOOD (to Dr. Francis Martin) and U.S. DOE Oak Ridge National Lab Plant-Microbe Interfaces project (Dr Francis Martin and Dr Claire Veneault-Fourrey). Collaborations to ensure ChipSEQ analysis will be developed.

Skills that the doctoral student will gain during the contract:

The PhD student will gain strong skills on fungal molecular biology and plant genetic transformation. These parallel competences are a critical asset for future specialist working on plant-microbes interactions. In addition, biochemistry tools and cell biology tools will be also used in the frame of the PhD. Specifically; he/she will develop methods such as Chip-SEQ, Yeast-two hybrid screens, *in planta* co-immunoprecipitation.

Publications of the research group on the topic (max. 5):

1. Plett JM, Daguerre Y, Wittulsky S, Vayssieres A, Deveau A, Melton SJ, Kohler A, Morrell-Falvey J, Brun A, Veneault-Fourrey C, Martin F (2014) The effector MiSSP7 of the mutualistic fungus *Laccaria bicolor* stabilizes the Populus JAZ6 protein and represses JA-responsive genes. PNAS 111: 8299-8304

2. C Veneault-Fourrey et al., Genomic and transcriptomic analysis of Laccaria bicolor CAZome reveals insights into polysaccharides remodelling during symbiosis establishment. Fungal Biol. Gen., DOI:10.1016/j.fgb.2014.08.007 (2014).

3. J.M. Plett, Khachane A, Ouassou M, Sundberg B, Kohler A, Martin F, Ethylene and jasmonic acid act as negative modulators during mutualistic symbiosis between *Laccaria bicolor* and *Populus* roots. New Phytologist 202: 270-286 (2014).

4. C. Veneault-Fourrey, F. Martin (2011) Mutualistic interactions on a knife-edge between saprotrophy and pathogenesis. Current Opinion in Plant Biology 14: 444–450.

Research topic 4

Intra-annual dynamics of wood formation and carbon sequestration in conifer and deciduous temperate forests

Research Unit:

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Supervisors of the PhD thesis:

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General aims and state of the art:

Plant tissues annually sequester nearly half of the 120 Pg of carbon assimilated by photosynthesis (Lal 2008), and thus contribute to uptake 15% of anthropogenic CO_2 emissions (Pan *et al*. 2011). Most of the carbon is sequestered into the wood, which acts as a long-term storage pool — representing about 80% of the terrestrial biomass (Lal 2008). Biomass accumulation in northern hemisphere woody plants closely follows the seasonal cycles, with modifications that can strongly impact on the global carbon cycle (Piao et al. 2008, Zhao & Running 2010). However, the seasonal dynamics of woody biomass accumulation remains poorly quantified, limiting our understanding of the terrestrial carbon cycle, and particularly its sensitivity to on-going climate change.

In a very recent study (Cuny *et al. in prep.*), we found a consistent delay of about one month between the increase of radial size and carbon sequestration in tree stems, all along the growing season. These results are based upon three years of weekly observations of xylem increase in size and in carbon content, for three temperate mature mixed conifer forests (containing silver firs, Norway spruces, and Scots pines), spread along an altitudinal gradient in the Vosges mountains. A comparison of these detailed quantifications with a global dataset of xylem phenology in conifers revealed that such a lag is a common feature in Temperate, Boreal, Alpine, and Mediterranean forest biomes of the northern hemisphere. Moreover, we discovered that stem size increase closely matches photoperiod cycle, whereas woody carbon sequestration clearly follows the seasonal course of temperature. Because of this differential coupling with environmental factors, we may expect that the predicted change in the annual cycle of temperature (Stine *et al.* 2009) lead to shift the phasing between growths in size *vs*. in biomass. These results challenge widely applied definitions of growth and demonstrate that the seasonal dynamics of woody biomass accumulation cannot be simply inferred from stem size measurements (Barford *et al*. 2011, Etzold *et al*. 2011, Gough *et al*. 2008). Furthermore, this study has provided new insights into the long-term terrestrial carbon storage (Lal 2008), with important implications on the interactions between carbon cycle and climate changes.

Because of the inner dynamics of the xylogenesis process, a temporal lag between stem size increase and woody carbon sequestration is also expected for angiosperm species, which compose the temperate deciduous broadleaf forests, the most widespread biome in temperate zone (Woodward 1987). Despite its important implication on the understanding of the carbon cycle, to date, no quantification of this potential lag is available for angiosperms — which represent a much more difficult case than conifers because of their complex wood anatomy. Moreover, our first pioneer work on conifers was mainly focused on pinpointing the lag between size and biomass stem growth dynamics, along with quantifying their relationships with environmental factors (Cuny *et al. in prep*). However, to understand the functional meaning of the evidenced lags, a much more integrated approach is required.

In this PhD thesis project we will investigate three contrasted tree species: European beech, sessile oak, and Aleppo pine, growing in three historical flux tower sites: Hesse, Barbeau, and Font-blanche. The main objective of the PhD Thesis will be to describe the intra-annual dynamics of wood formation and carbon sequestration of these tree forest stands and to explore its relationship with internal and environmental factors.

Specific research topic:

In order to better understand the process of carbon sequestration into stem wood, along with its environmental determinants, we want to dynamically quantify the flux of carbon, from its capture into the leaves, to its final destination into the wood, for three contrasted couples of species and environmental conditions (European beech in Hesse, sessile Oak in Barbeau, and Aleppo Pine in Font-Blanche). So in this PhD project, we propose, for these three couples: (1) to describe precisely the intra-annual dynamics of wood formation, (2) to quantify accurately the seasonal dynamics of net ecosystem exchange, gross primary productivity, net primary productivity, non-structural carbon reserves, and finally structural carbon accumulation into the wood (structure and composition); (3) to compare the seasonal dynamics of the carbon fluxes, and compute dynamic ratios of carbon transfer between the different carbon pools; (4) to relate the seasonal dynamics of carbon fluxes with the seasonal course of environmental factors.

Capitalizing on the experience we gained from recent pilot studies conducted in the Fontainebleau forest and in the recently set up flux tower site of Montiers, and on recent collaborations developed on this subject, samplings will be performed weekly on seven dominant trees for the tree studied sites over the growing season 2015, 2016 and 2017. The tree studied species are biological models for the tree types of tree-ring structure encountered in temperate forests.

Novelty and relevance to the research project of the team:

This thesis project is build around a common scientific question shared by all the members of the thesis comity. Partner's teams historically rollout different methodologies and scales of observations to tackle this question. Joining our forces together will allow us to see at the same time greater details and the broad picture, answering the question a lot more satisfactorily.

This project is based on collaboration between partners presenting strong and complementary expertise in: (1) forest ecosystem photosynthesis and respiration quantification — Bernard Longdoz (EEF, INRA Nancy-Lorraine); process-based modelling of forest stand productivity — Nicolas Delpierre (ESE, Paris-Sud University); tree growth and wood formation monitoring (Cyrille Rathgeber & Julien Ruelle (LERFoB and Xylosciences plateform, INRA Nancy-Lorraine); non-structural carbon measurements — Dominique Gérant (EEF, Lorraine University), and finally wood isotopic analysis — Stéphane Ponton (EEF, INRA Nancy-Lorraine).

Based on this expertise, the supervising board will help the PhD student to address questions 1 to 4, which have never been answered for angiosperms.

Potential impact for the scientific discipline and the society:

This PhD project addresses timely environmental and societal challenges such as the impact of global changes on carbon cycle, forest productivity, and wood quality. The project handles a far-reaching scientific question: what is the seasonal dynamics of carbon fluxes into a forest ecosystem, from the capture by the canopy to sequestration into the wood. We believe that this project will obtain original results of great importance for the scientific community, which will be communicated in international conferences, and published in top international peer-review journals.

Available equipment / experimental support / associated research projects:

The spatiotemporal design of the sampling will be of crucial importance for this project, because we want to: compare estimations made at the ecosystem level (NEE, GPP, etc.), stand level (NPP, etc.), and tree level (NSC, WSC, $\delta^{13}C$, etc.); To build up the needed innovative sampling design, we will rely on the experience gained in a pilot study conducted in 2014 on the Montiers (Meuse, France) flux tower site.

Moreover, in the framework of the WoodCap Project (labex ARBRE), we have started sampling at Hesse site during the 2015 growing season. At Hesse site, sensors are collecting automatically half-hourly soil and meteorological data (air, canopy and soil temperatures, precipitation, global, net and photosynthetic active radiations, wind speed, soil conduction, water table depth, air and soil humidity, etc.), as well as vegetation indexes

(NDVI[1], PRI[2]), and canopy digital photography (1 per day). The Net Ecosystem Exchange (NEE) is deduced directly from the post-processing of the eddy covariance raw measurements (Longdoz *et al*. 2008). The gross primary productivity (GPP) is calculated by subtracting the ecosystem respiration from NEE. GPP represents the amount of atmospheric carbon captured by leaf photosynthesis. A large part of the carbon fixed by photosynthesis is released back in the atmosphere by autotrophic respiration (Ra). In this project, we will use CASTANEA — a process-based model design to compute carbon and water stocks and fluxes in pure forest stands — in order to simulate the seasonal course of net primary productivity (NPP = GPP – Ra). We will follow carbon allocation to primary and secondary growth on the seven selected trees, using phenocams to monitor leaf phenology and shoot growth, and dendrometers to monitor stem size variations. For each tree, small wood samples (microcores including the phloem, cambial zone, the forming ring and some previous rings) will be collected weekly on tree stem, from April to November 2015. Microcores will be treated using Xyloscience platform facilities, to quantify the magnitude and seasonal dynamics of carbon sequestration into stem wood with high temporal accuracy. A second set of microcores will also be taken weekly in order to quantify the seasonal dynamics of the chemical composition of the carbon compounds using PTEF platform facilities. The carbon isotope composition ($\delta^{13}C$) will be measured on the WSC extracted from the microcore samples using PTEF platform facilities. We propose to use this isotopic signature to investigate further the origins of the carbon sequestered into wood.

Similar monitoring will be also started at the two other partner's flux tower sites: Font-Blanche and at Barbeau.

Skills that the doctoral fellow will gain during the contract:

Technical skills: field survey, laboratory work, microscopic observations, and image analysis.

Methodological skills: data management and data mining, data analysis in R (multivariate analysis, logistic regression, mixed models, general additive models and sensitivity analysis).

Cognitive skills: grasping the objectives of the project and being able to use the results to progress in the understanding of the process of wood formation and the functioning of the forest ecosystems. Being able to write and publish the results in the best journals of plant science and ecology.

Other: good English, managing multidisciplinary studies and developing human skills.

Five publications of the research group on the topic:

Cuny, H. E., Rathgeber, C. B. K., Kiessé, T. S., Hartmann, F. P., Barbeito, I., & Fournier, M. Generalized additive models reveal the intrinsic complexity of wood formation dynamics. *Journal of Experimental Botany 64,* (2013).

Cuny, H. E., Rathgeber, C. B. K., Frank, D., Fonti, P. & Fournier, M. Kinetics of tracheid development explain conifer tree-ring structure. *New Phytologist* (2014).

Delpierre N, Soudani K, Francois C*, et al.* Exceptional carbon uptake in European forests during the warm spring of 2007: a data-model analysis. *Global Change Biology* 15, 1455-1474 (2009).

Longdoz B., Gross P., Granier A. Multiple quality tests for analysing CO_2 fluxes in a beech temperate forest. Biogeosciences, 5, 719–729 (2008).

Michelot, A., Simard, S., Rathgeber, C., Dufrêne, E., & Damesin, C. (2012). Comparing the intra-annual wood formation of three European species (*Fagus sylvatica*, *Quercus petraea* and *Pinus sylvestris*) as related to leaf phenology and non-structural carbohydrate dynamics. *Tree Physiology*, *32*(8), 1033–1045.

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[1] *NDVI: Normalized difference vegetation index*
[2] *PRI: Photochemical reflectance index*

Etzold, S. *et al.* The carbon balance of two contrasting mountain forest ecosystems in Switzerland: similar annual trends, but seasonal differences. *Ecosystems* **14**, 1289-1309 (2011).

Gough, C., Vogel, C., Schmid, H., Su, H.-B. & Curtis, P. Multi-year convergence of biometric and meteorological estimates of forest carbon storage. *Agricultural and Forest Meteorology* **148**, 158-170 (2008).

Lal, R. Sequestration of atmospheric CO_2 in global carbon pools. *Energy & Environmental Science* **1**, 86-100 (2008).

Pan, Y. *et al.* A large and persistent carbon sink in the world's forests. *Science* **333**, 988-993 (2011).

Piao, S. *et al.* Net carbon dioxide losses of northern ecosystems in response to autumn warming. *Nature* **451**, 49-52 (2008).

Zhao, M. & Running, S. Drought-induced reduction in global terrestrial net primary production from 2000 through 2009. *Science* **329**, 940-943 (2010).

Stine, A. R., Huybers, P. & Fung, I. Y. Changes in the phase of the annual cycle of surface temperature. *Nature* **457**, 435-440 (2009).

Research topic 5

Understanding detoxification systems developed by wood-decaying fungi and potential application to assess the natural durabiloity of wood

Research Unit:

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General aims and state of the art:

The natural durability of wood is defined as its ability to biotic and abiotic degradation agents. Microorganisms and especially wood decaying fungi are among the most important agents involved in wood degradation. The wood degradation strategies developed by fungi involve either selective enzymatic degradation of wood polymers (Van den Brink and de Vries, 2011; Martínez et al., 2005) or non-enzymatic Fenton and Fenton-like reactions (Arantes et al., 2012; Tanaka et al., 1999). Additionally to these systems to degrade wood polymers, fungi are also known to develop strategy for the detoxification of wood extractives considered to be responsible for the natural durability of wood. Among the main categories of wood-rotting fungi, white-rot and brown-rot fungi and their enzymes naturally involved in wood extractives degradation, among which lipases and laccases, are being increasingly used in a variety of biotechnological applications (Singh, 2014). More recently, it has been revealed that in complement to the extracellular machinery of degradation, intracellular antioxidant and detoxification systems contribute also to the lignolytic capabilities of fungi, presumably by preventing cellular damages and maintaining fungal health (Thuillier et al., 2014). Focusing on these systems, glutathione transferases have been shown to interact with extracts of different wood species indicating more or less important substrate specificity according to the nature of extractives present in these mixtures. On the other side, extractives have been reported to be directly connected to wood natural durability (Huang et al., 2009; Neya et al., 2004; Nzokou et al., 2005; Antwi-Boasiako et al., 2010; Kirker et al. 2009).

Even if the amounts and the nature of extractives vary greatly according to intra and interspecific parameters, these latter ones belongs generally to more or less similar chemical families. The main families of extractives can be summarized as follows (Fengel and Wegener, 1989): fats, waxes and fatty acids, terpens and terpenoids and phenolic compounds including simple phenols, flavonoids, stilbenes, quinones, lignans and tannins…

Durability of heartwood impacts directly wood utilization for outdoor application, where the material is subjected to the attack of microorganisms. In this context, natural durability is not always sufficient to ensure full protection of the construction during its service life justifying the utilization of the more durable wood species. However, wood natural durability may vary within same wood specie, which may be at the origin of durability problems during wood utilization (Aloui et al., 2004; Guilley et al., 2004). Currently, methods used to evaluate wood natural durability involve exposure of small wood samples to different test fungi and evaluation of mass losses after several weeks of incubation. For a better utilization of the forest resource, it is seems necessary to be able to characterize easily the properties of the material justifying the development of alternative methods allowing a more rapid screening of durability.

In this context, the objectives of this proposal are to develop new methods based on interactions of wood extractives and selected enzymes among which glutathione transferases able to qualify rapidly wood durability. For this purpose, we plan to investigate the effect of different molecules present naturally in wood extracts on different glutathione transferases allowing characterization of functional role of these enzymes. In parallel, we will test the effects of wood extracts on these enzymes in order to correlate wood durability measured using classical weight loss methods to inhibition of GST activities.

Specific research topic:

From a fundamental point of view, the specific research question will concern the elucidation of the functional role and the substrate selectivity of GST with respect to the diversity of molecules contained in wood extracts.

From a more applied point of view, the second specific research question will concern the possibility to use enzymatic tests to predict wood durability based on the hypothesis that wood decay will start after the detoxification step necessary for the fungus to colonize the material.

Novelty and relevance to the research project of the team:

The research project will combine multidisciplinary approaches at the frontiers of molecular biology, biochemistry and chemistry. Association of competences of LERMAB and IAM will offer a unique framework to carry out such a multidisciplinary project. Its novelty lies in the fact to develop molecular biological tools to assess wood durability but also to identify molecules of interest in crude extracts allowing identification of molecules of interest for further chemical valorization.

Potential impact for the scientific discipline and the society:

The potential impact for the society and scientific community are the following:

- improve wood utilization through better understanding of its properties in relation with its natural variability,

- increase the knowledge in the field of wood biodegradation mechanisms

- permit an easy identification of molecules of interest in crude extracts on the basis of simple enzymatic tests,

- develop biotechnological applications in the field of water treatment, biomass preconditioning for bio-refinery applications or de-pollution.

Available equipment / experimental support / associated research projects:

Different equipments necessary to characterize wood durability and to identify composition of wood extractives as well as to follow modification of extractives after enzyme exposure are available at LERMAB. This include microbiological hood, climatic chambers, sterilization autoclave, LC-MS, GC-MS….

The equipment necessary to produce and characterize the recombinant proteins is available in the IAM department. The functional characterization of proteins of interest will be performed after production and purification of recombinant proteins in *E. coli*. To date, we have produced more than 20 GSTs from various organisms without major problems to obtain high amounts of folded and functional proteins. The different catalytic and binding properties of the GSTs will be tested (alkylation, arylation, addition to isocyanates, transacylation, hydroperoxide reduction, steroid isomerisation, deglutathionylation…) using usual spectrometric or fluorogenic detection. We have developed competition tests using fluorescent probes and also a fluorescence-based thermal stability method to test the interactions between wood extractives and GSTs.

Skills that the doctoral fellow will gain during the contract:

The doctoral fellow will acquire competences in analytical chromatographic techniques necessary to characterize modification of extractives exposed to GST, but also to identify the main components of natural wood extracts in order to study structure activity relationship of enzymes. In a similar time, it will acquire expertise in the field of protein production and characterization.He will also acquire more technical knowledge in the field of degradation and wood protection including classical microbiology.

Five publications of the research group on the topic:

- Mathieu, Y., Prosper, P., Bué, M., Dumarçay, S., Favier, F., Gelhaye, E., Gérardin, P., Harvengt, L. Jacquot, JP., Lamant, T. , Meux, E., Mathiot, S., Didierjean, C., Morel., M. (2012) Characterization of a Phanerochaete chrysosporium Glutathione Transferase Reveals a Novel Structural and Functional Class with Ligandin PropertiesJournal of Biological Chemistry, 287: 39001-39011

- Meux, E., Morel, M., Lamant, T., Gérardin, P., Jacquot, JP., Dumarçay, S., Gelhaye., E. (2013) New substrates and activity of Phanerochaete chrysosporium Omega glutathione transferases. *Biochimie*, 95(2):336-46

- Antioxidant activities, total phenolic contents and chemical compositions of extracts from four Cameroonian woods: padouk (*Pterocarpus soyauxii* Taubb), tali (*Erythrophleum suaveolens*), moabi (*Baillonella toxisperma*), and movingui (*Distemonanthus benthamianus*). (2013) Tchinda Saha, JB., Abia, D., Dumarçay, S., Kor Ndikontar, M., Gérardin, P., Ngamveng Noah, J., Perrin, D. Industrial Crops and Products, 41, 71- 77

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- Guilley E, Charpentier J P, Ayadi N, Snakkers G, Nepveu G, Charrier B (2004) Decay resistance against Coriolus versicolor in Sessile oak (Quercus petraea Liebl.): analysis of the between-tree variability and correlations with extractives, tree growth and other basic wood properties. Wood Science and Technology 38(7): 539-554

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