Young scientist contract offered by Inra within the doctoral school RP2E (Université de Lorraine)
Call for applications - March 2014

Inra and the Doctoral School RP2E offer during 2014 one "Contrat Jeune Scientifique" (Young Scientist Contract) for 3 years (duration for completion of a PhD) followed by two years of postdoctoral research abroad. Research will take place at Nancy (France). Candidates may choose among the four topics proposed this year:

Topic 1
From leaf to whole plant: morphological, physiological and molecular determinants of transpiration and water use in poplar trees
Topic 2
Understanding the molecular dialogues within forest soil microbial communities and investigating their impact on plant health and growth
Topic 3
Functional characterization of a chloroplast-targeted candidate effector of the poplar rust pathogen *Melampsora larici-populina*: could the rust fungus hijack chloroplastic functions to achieve infection?
Topic 4
Functional variability of the detoxification system in lignolytic fungi

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 (350 000 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 (presid@nancy.inra.fr) before Monday May 12th, 2014.

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 2014). 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: presid@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

From leaf to whole plant: morphological, physiological and molecular determinants of transpiration and water use in poplar trees

Research Unit:

UMR EEF 1137 Forest Ecology and Ecophysiology (Centre Inra de Nancy-Lorraine, Université de Lorraine)

Supervisors of the PhD thesis:

Dr Didier LE THIEC (Research Director) Accreditation to supervise research (HDR)
Tél: +33 (0)3 83 39 40 98
Mail: le_thiec@nancy.inra.fr

Dr Oliver BRENDEL (Researcher) Accreditation to supervise research (HDR)
Tél: +33 (0)3 83 39 41 00
Mail: brendel@nancy.inra.fr

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 *Wi* and stomatal conductance and stomatal density; the 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 objectives are: (i) to identify the parameters (leaf gas exchange, leaf anatomy, dynamics of stomatal responses, whole plant allometry, etc...) that are important in upscaling water-use efficiency from leaf to whole tree and from greenhouse to planted trees; (ii) to identify the molecular processes in guard cells related to the observed differences among genotypes; (iii) to use a modelling approach to predict whole plant *TE* from leaf gas exchange parameters and assess the differences among genotypes.

Two euramerican and two black poplar genotypes known to differ in WUE will be used in field and greenhouse environments. Two euramerican genotypes have been chosen (Carpaccio and I214), that differ in growth, leaf area, leaf anatomy, stomatal conductance and carbon discrimination (Monclus et al., 2006). Official recommendations for plantations of these genotypes differ for soil water availability, with I214 less indicated for drought prone situations (http://agriculture.gouv.fr). Two black poplar genotypes (N38 et 6J-29) have been chosen among those used in the European project WATBIO; data analyses are in progress and first results suggest that N38 displays larger and thicker leaves, lower stomatal conductance and transpiration and higher water use efficiency.

The PhD will be integrated into the European project Watbio, which aims at understanding the effects of drought on the development of tissues and organs in poplar. The project will provide transcriptomic and metabolomic data on leaf, wood and root growth as well as ecophysiological data on traits related to water use efficiency.

The PhD project will be structured around several specific questions:

- Are the differences in stomatal rapidity and sensitivity among poplar genotypes changing between measurements in controlled and in field conditions? Which environmental variables are the drivers for such differences (like light intensity and quality, VPD, leaf temperature and soil water content)?
- How do stomatal dynamics 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)?
- Are the processes controlling whole plant transpiration efficiency similar to those controlling leaf water use efficiency?
- What is the impact of potential genortypic differences in carbon allocation and nocturnal transpiration on Φ_w and/or Φ_c, and thus on upscaling from leaf water use efficiency to whole plant transpiration efficiency?

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 Université de 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 will be selected according to their tolerance to drought (from previous studies, such as the European project WATBIO). 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). These trees will be planted in the nursery at Inra Champenoux in 2014 and will be subjected to a reduced water input by rain exclusion. We have analytical tools 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 researchers supervisors.

Skills that the doctoral fellow will gain during the contract:

The supervisors are committed to train the student to build hisPhD project and his professional project. Cognitive skills that will be acquired during the training 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 leaf 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 the 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 in ecologicazl process modelling, to participate to the thesis committee.

Thesis committee (*Suggested composition; may evolve with time)*

Thierry Simonneau (DR, Inra Montpellier)
Franck Brignolas (Prof, Orléans)
Hendrik Davi (CR Inra Avignon)
Koen Kramer (researcher, Wagenignen University)

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*, doi: 10.1111/pce.12293.

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.

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.

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.

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Fichot R. 2010. Variabilité structurale et fonctionnelle du xylème et plasticité en réponse à la sécheresse chez le peuplier. Université d'Orléans, Soutenue le 23/06/2010, 194p

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Lange OL, Losch R, Schulze ED, Kappen L. 1971. Responses of stomata to changes in humidity. *Planta* 100 : 76-86.

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 2

Understanding the molecular dialogues within forest soil microbial communities and investigating their impact on plant health and growth

Research Unit:

UMR 1128, Dynamique des génomes et adaptation microbienne (DynAMic)

Supervisors of the PhD thesis:

Bertrand Aigle, Pr, UMR 1128 DynAMic
Tél: +33 (0)3 83 68 42 05
Mail: bertrand.aigle@univ-lorraine.fr

Pierre Leblond, Pr, UMR 1128 DynAMic
Tél: +33 (0)3 83 68 42 07
Mail: leblond@nancy.inra.fr

General aims and state of art:

Among forest soil ecosystems, including environments such as rhizosphere, the bacteria of the *Streptomyces* genus are expected to play a major role in the structuring and dynamics of the microbial communities. *Streptomyces* are well known for their ability to produce a wide range of secondary metabolites (also called natural products) including antibiotics. Although it has been assumed for a long time that antibiotics play their main role in antibiosis (as 'molecular weapons'), more and more recent data however underline their key impact in cell-cell communications and lead to a more complex and subtle definition of their role in natural ecosystems. Other *Streptomyces* natural products, e.g. siderophores, are expected to influence the behaviour of the microbes sharing the same ecological niche. Plants themselves are also influenced by *Streptomyces*. It has been shown that their secondary metabolites elicit the hypersensitive response of plants to pathogens or stimulate mycorrhiza formation between symbiotic fungi and plants.

The general aim of the project is to acquire insight into the impact of the dialogues between microorganisms on their behaviour, on their response to environmental changes as well as on the structure of the microbial communities. In the longer term, the goal is to understand how specific dialogues established between microbes influence plant development and how plants themselves take part in these metabolic exchanges.

Specific research topic:

The proposed project aims at deciphering the dialogues between the bacteria *Streptomyces* and their microbial neighbours *i.e.* the signalling molecules, the signal transduction pathways and the targeted metabolic pathways, with a particular emphasis on the role of the antibiotics in these exchanges. We aim at understanding how the *Streptomyces* species influence microbial communities of the rhizosphere and how this can impact plant (in particular tree) health and growth.

The microbial partners will be originating from rhizosphere at the micro-niche level (soil grain) in which the isolated microorganisms are likely to have experienced biotic and functional interactions. Microbial community composition will be analysed by metagenomic approaches (deep sequencing). Bacterial and fungal samplings will be obtained through classical isolation, and further typified by rDNA sequencing. Analyses of the interactions between a *Streptomyces* strain selected as a "model" and microorganisms of the collection (bacteria and fungi) will first be performed by pairwise cultures on media of growing complexity (up to a mimic of the natural environment) and by

observation of growth and morphology of the partners and bioactivity tests. For co-cultures showing specific phenotypes (e.g. cooperation, stimulation of growth...), the microbial dialogues will be studied at a global and spatio-temporal scale by Imaging Mass Spectrometry (IMS) and nanoDESI mass spectrometry. The metabolites of *Streptomyces* and of its partners whose biosynthesis is influenced by the interaction and could therefore play a role in the dialogue will be characterized further. In parallel, the genome of the model *Streptomyces* will be sequenced to identify the secondary metabolite biosynthetic gene clusters and transcriptional analyses will allow identification of clusters whose expression is modulated in the presence of the partner as well as genes encoding the molecular actors potentially involved in the transduction of the communication signals. A similar approach will be adopted out with the microbial partners. Tripartite interactions will be also considered such as *Streptomyces* - bacterial partner - fungi.
Microcosms mimicking soil microbial communities (bacteria, fungi) will be performed to assess the impact of the resulting cross-talk on plant/tree parameters such as root development, plant growth…Reciprocally, the effect of the plant on the interaction between *Streptomyces* and its microbial partners will be studied.

Available equipment / experimental support / associated research projects:

Equipment for transcriptional analysis (real-time PCR, microplate reader for fluorescent reporter genes...), microbiology and classical molecular microbiology are available in the laboratory. The laboratory also has access to the microscopic, genomic and metabolomic (including HPLC, LC-MS...) platforms on site. Collaborations have been initiated with Dr Aurélie Deveau (Inra, Nancy) on the microbial ecology part of the project and with Dr Pieter Dorrestein (UCSD, USA) for the global analysis of metabolic exchange by spectrometric analysis (IMS and nanoDESI).

Skills that the doctoral student will gain during the contract:

The proposed project will allow the doctoral student to gain skills in molecular microbiology, integrative biology (genomics, transcriptomics and metabolomics) as well as in microbial ecology thanks to the collaboration with Dr Aurélie Deveau (UMR 1136, IaM). He/she will also be trained for chromatographic analyses (HPLC, LC-MS, IMS...) as the project may lead to the identification and chemical characterization of novel secondary metabolites.

The student will be fully involved in the development of the strategy of the project and of new research directions that may occur during the thesis. He/she will be expected to become autonomous relatively quickly.

Finally, the doctoral student will acquire open-mindedness through the collaborations he/she will have to es tablish during his /her PhD. All these qualities will allow him/her to apply for different positions after the PhD in molecular ecology and forestry research areas or in biotechnology.

The lab

Our lab is a mixed unit between the University of Lorraine and Inra (the French National Institute for Agricultural Research) with 23 members. It is part of a Research Federation which locally groups together 13 research units and which has developed several technical platforms to which we have an easy access.

The lab has collaborations with members of this Federation and also with colleagues in other fields (chemistry, computing sciences...).

Research topic 3

Functional characterization of a chloroplast-targeted candidate effector of the poplar rust pathogen *Melampsora larici-populina*: could the rust fungus hijacks chloroplastic functions to achieve infection?

Research Unit:

UMR 1136 Interactions Arbre-Microorganismes / Tree-Microbe Interactions Department (Centre Inra de Nancy-Lorraine, Université de Lorraine)

Supervisors of the PhD thesis:

Sébastien Duplessis, INRA Research Director (HDR)
UMR 1136 IAM, EcoGenomic Team (Centre Inra de Nancy-Lorraine)
Tél: +33 (0)3 83 39 40 13
Mail: sebastien.duplessis@nancy.inra.fr

Arnaud Hecker, Junior Lecturer, Chair of Excellence Inra
UMR 1136 IAM, Stress Response and Redox Regulation (Université de Lorraine)
Mail: arnaud.hecker@univ-lorraine.fr

General aims and state of the art:

The last decade has been rich in discoveries in the field of molecular phytopathology and has been marked by conceptual syntheses on the molecular bases of plant-pathogens interactions, proposing an unified description of the plant immune system (Dodds and Rathgen 2010). The current paradigm establishes that a parasite can be detected at two levels by receptors of the plant immune system. A first level is via the recognition of conserved pathogen/microbial molecular patterns (PAMPs) by cell-surface receptors (PAMPs Triggered Immunity). Pathogens can overcome such detection by delivering effectors in the plant host cells that will interfere or suppress this general recognition (Effector-Triggered Susceptibility). In turn, plants develop a second level of resistance that is set upon the specific recognition of pathogen effectors by plant cytoplasmic receptors (Effector-Triggered Immunity). When the plant is unable to detect the pathogen and efficiently trigger immune responses at these two layers, the microbe establishes a compatible interaction that lead to the expression of the disease. In the past decade, a few resistance receptors and effectors have been characterized (Dangl et al. 2013). Their identification and description in different pathosystems remain crucial in order to define new means to fend-off diseases in cultures and ecosystems. The characterization of parasitic effectors is a very active field. After the sequencing of many pathogens genomes, several questions are still opened: How pathogens manipulate the plant immune system? Which effectors are delivered into the host cells? How and where these effectors are addressed inside the host cell? How the host is able to detect these effectors? How these effectors and their recognition counterpart evolve in natural ecosystems and in managed agrosystems?

Specific research topic:

The rust fungus *Melampsora larici-populina* is responsible for the poplar leaf rust disease that causes important damages in plantations worlwide. In the past years, the UMR 1136 IAM has made considerable progress in the description of molecular determinants of this tree-pathogen interaction, particularly through the angle of genomics. The sequencing of the genomes of both the host plant *Populus trichocarpa* (Tuskan et al. 2006) and *M. larici-populina* (Duplessis et al. 2011a) has led to the identification of repertoires of poplar immune receptors (Kohler et al. 2008) and of poplar rust candidate effectors (Hacquard et al. 2012). Transcriptomic analyses have narrowed the list

of candidate effectors based on their specific expression profiles during host-infection (Duplessis et al. 2011b). More recently, RNA-Seq studies conducted in the frame of the ANR POPRUST project identified a cluster of host plant genes highly regulated during the compatible interaction, corresponding in majority to chloroplastic and photosynthesis components (Duplessis et al. unpublished data).

In the past five years and in the frame of a former CJS project (Benjamin Pêtre, PhD at INRA Nancy and Université Lorraine and post-doc at The Sainsbury Laboratory, TSL, Norwich, UK) we have set different approaches aimed at characterizing selected effector candidates. A high-throughput phenotyping screen based on the transient expression of candidate effectors in the model plant *Nicotiana benthamiana* has allowed to detect some candidates presenting phenotypes in the plant cell, such as particular subcellular localisation and specific interaction with plant proteins (Petre et al. unpublished data). We now have a short list of promising candidate effectors that remain to be characterised. One particular candidate effector localises in the chloroplast matrix and co-immunoprecipitates with chloroplast-resident proteins. We named this candidate effector CTCE1 for Chloroplast-Targeted Candidate Effector 1.

To date, few fungal effectors have been characterized and none have been shown to target chloroplasts. The project could reveal novel fungal virulence strategies aimed at manipulating chloroplasts and/or photosynthetic process, thereby providing further insights into how these pathogens successfully infect their hosts.
The aim of the PhD project will be to further characterize CTCE1. For this purpose the doctoral fellow will use different approaches such as:

- Transient expression in *N. benthamiana* and in poplar leaves i) to confirm localisation in poplar cells, ii) to determine whether the chloroplast localisation is mediated by a transit peptideand and iii) to perform dual co-immunoprecipitations to validate CTCE1 candidate plant protein partners
- Stable transgenic poplars expressing CTCE1 will be generated and their phenotype will be scrutinized, particularly parameters related to chloroplastic functions (e.g. photosynthesis)
- Recombinant proteins will be produced in *Escherichia coli.* Purified proteins will be used for in vitro co-immunoprecipitation in order to validate poplar interactants. The structure of the effector will possibly be resolved (cristallography, RMN). Finally, purified proteins will allow designing specific antibodies against CTCE1 to perform time-course expression profiling by Western Blot and immunolocalization during poplar infection
- The recombinant protein will be assayed by infiltration in a large panel of poplar clones in order to test whether immune components could target the effector (detection of Hypersensitive Response)
- Finally, homologous CTCE1 genes will be searched in *M. larici-populina* and other close rust fungal species.

Novelty and relevance to the research project of the team:

The study of evolution and mechanisms of the *Populus-Melampsora* pathosystem is a transversal theme of the UMR 1136 IAM that brings together scientists from three different teams with a large panel of competences from genomics and population genetics to biochemistry and molecular biology. In the past five years, the research conducted has established the *Populus-Melampsora* pathosystem as a model system in tree pathology. The team has also engaged collaborations with external teams and set approaches with the pathosystem as well as in heterologous systems to perform wide-screen of *Melampsora* candidate effectors in order to identify *bona fide* effectors. The main aim of the CJS is to finalize this long term effort to tackle down rust fungus effectors, by conducting the functional characterization of the most promising selected candidate. Although, other interesting candidates have been identified, the localization in the chloroplast is very singular. The fact that transcriptomic studies in the host plant have suggested the manipulation of the chloroplast and/or photosynthesis by the fungal pathogen, we see here a strong potential for bridging the research conducted in the UMR IAM with other teams on the Centre Inra Nancy-Lorraine (e.g. tree physiology). We think that such a CJS project will provide ground for a larger integrative biology project.

Potential impact for the scientific discipline and the society:

Although we have now a better view of bacterial effectors, until now, only a handfull of effectors from eukaryotic plant filamentous pathogens have ben described and characterized. For some, the sub-cellular localisation is known and putative targets have been identified. Only in a few cases, the function is determined and characterized. Plethora of effectors have been described in various pathosystems and the field of effector research is highly active.

Current researches provide a great knowledge on the functioning of the molecular plant-pathogen interactions and crucial hints to define proper strategies for long-term management of resistance to pests. Plant-pathogen interactions in forest trees and forest ecosystems are even more complex to decipher considering that trees are non-amenable plant models compared to annual species such as *Arabidopsis thaliana* and *N. benthamiana*..The *Populus-Melampsora* pathosystem is not only a model in forest pathology, the disease also strongly impacts plantations of poplar trees worlwide and is a real concern for poplar breeders in Northern Europe.The disease is particularly marked by decreased photosynthetic ability and intense defioliation at the end of the vegetative season in plantations. Studying by which mechanisms the fungus specifically targets functions in poplar chloroplasts will be particularly important in this regard and to understand this component of the tree-fungus interaction.

Available equipment / experimental support / associated research projects:

All equipments required to perform the study (S2 greenhouse, indoor phytotronic chambers, microscopy devices, molecular biology facilities for DNA, RNA and protein manipulations, bioinformatic support) are available at one or the two sites of the proposing researchers (Centre Inra de Nancy-Lorraine and Université de Lorraine).

The CJS proposal is related to the ongoing collaborative project IntegraRust (2013-2015) in the frame of the Labex ARBRE and will benefit from outcomes of the POPRUST ANR project, and from the work of the former CJS (Benjamin Pêtre). The PhD project will be central to an ANR project that will be submitted to the 2014 call.

Skills that the doctoral fellow will gain during the contract:

The doctoral fellow will gain knowledge in different scientific disciplines such as molecular phytopathology, genomics, microbiology and plant sciences. The doctoral fellow will develop technical skills in a large panel of approaches from cell and molecular biology,molecular engineering, microscopy, genomics and transcriptomics, and biochemistry.

It is expected during the frame of the project to develop physiology and ecophysiology analyses in collaboration with external teams. This will contribute to broaden the doctoral fellow skills.

Thesis committee (suggested members):

It is important for the CJS project to gather a specialized panel on the topic of effectors and their functional characterization. We propose to bring together scientists working on different pathogens (fungi, nematods and oomycetes). Crossing perspectives will be of great interest to identify potential pitfalls. Also, we would like to include colleagues with experience with tree molecular engineering as well as with chloroplast organelles.

- Isabelle Fudal (Inra Versailles-Grignon) is a young scientist developing the functional study of *Leptosphaeria maculans* effectors. She uses a set of approaches similar to those that the CJS fellow will apply to *Melampsora*.
- Claire Veneault-Fourrey (Inra Nancy-Lorraine) is a junior lecturer at Université de Nancy, member of the UMR 1136 IAM, studying effectors of the mutualistic fungus *Laccaria bicolor.*
- Bruno Favery (Inra Antibes) is a senior scientist at Inra Sophia-Antipolis actually developing functional aspects to study effectors of pathogenic nematods. He is particularly interested in the host components targetted by effectors.
- Benjamin Petre is a postdoc fellow at TSL working on the screening of *Melampsora* effectors in the frame of his CJS (until september 2014). He was pivotal to the previous *Melampsora* effectors project and he will bring a broad view on the topic to the new CJS candidate.
- Annabelle Déjardin (Inra Orléans) is a scientist working on physiology of wood development in trees. She has a long experience in molecular engineering of poplar trees.
- A scientist specialist of the chloroplast organelle (e.g. from the CEA IRTSV at Grenoble, France).

Five publications of the research group on the topic:

1. Duplessis et al. (**2011**) Obligate biotrophy features unraveled by the genomic analysis of rust fungi. ***PNAS***, **108**:9166-9171

2. Duplessis et al. (**2011**) Melampsora larici-populina transcript profiling during germination and time-course infection of poplar leaves reveals dynamic expression patterns associated with virulence and biotrophy. ***MPMI***, **24**:808-818

3. Duplessis S, Joly DL, Dodds PN (**2012**) Rust effectors. Chapter 7 In *Effectors in Plant-Microbes Interactions*, Martin F and Kamoun S (eds), Wiley-Blackwell, pp-155-193

4. Hacquard et al. (**2012**) A comprehensive analysis of genes encoding small secreted proteins identifies candidate effectors in Melampsora larici-populina (poplar leaf rust). ***MPMI***, **25**:279-293

5. Hacquard et al. (**2011**) The poplar-poplar rust interaction: insights from genomics and transcriptomics. ***Journal of Pathogens***, **doi:10.4061/2011/716041**

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Dangl et al. (2013) Pivoting the plant immune system from dissection to deployment. ***Science***

Dodds & Rathjen (2010) Plant immunity: Towards an integrated view of plant-pathogen interactions. ***Nature Rev Genet***

Kohler et al. (2008) Genome-wide identification of NBS resistance genes in *Populus trichocarpa*. ***Plant Mol Biol***

Tuskan et al. (2006) The genome of black cottonwood, *Populus trichocarpa*. ***Science***

Research topic 4

Functional variability of the detoxification system in lignolytic fungi

Research Unit:

The IaM research department (http://mycor.nancy.inra.fr/IAM/) is a joint Research Unit of Inra and Lorraine University that comprises ecologists, geneticists, microbiologists, physiologists, genomicists, and molecular and cell-biologists. The research programme of the IaM research Unit aims to unravel the role of belowground multitrophic interactions in the structure of microbial and plant communities in forest ecosystems as well as the role of these microorganisms in tree nutrition. IaM has an excellent track record using both protein biochemistry and genomics to decipher tree-microbe interactions. The Unit has specific expertise in the biochemistry of redox proteins, genomics, bioinformatics, metagenomics, microbial ecology, plant microbe-soil interactions and forest pathology. In this Unit, the 'Réponses aux stress et régulation rédox' team focuses its research primarily on the redox regulation mechanisms focusing on the structure-function relationship of antioxidant enzymes belonging to the thioredoxin, glutaredoxin, thiol peroxidase and methionine sulfoxide reductase families. It provided many mechanistic details for their catalytic cycle and mode of interactions. The current projects deals with the functional analysis of two classes of glutathione-dependent enzymes, glutaredoxins (Grxs) and glutathione transferases (GSTs). Another important part of the team research is to identify by comparative genomics the key gene networks controlling fungal adaptation to their ecological niches (wood, litter, roots). To date we have focused on various strains of *Trametes versicolor*, highlighting genomic and functional polymorphism. The functional characterization of the identified candidates will allow to better understand how the fungi adapt to local environmental pressures.

Supervisors of the PhD thesis:

Morel-Rouhier Mélanie, UMR IaM Interactions arbres Microorganismes, Team "Réponses aux stress et régulation rédox" Lorraine University
Tél: +33 (0)3 83 68 42 28
Mail: melanie.morel@univ-lorrraine.fr

Gelhaye Eric, UMR IaM Interactions arbres Microorganismes, Team "Réponses aux stress et régulation rédox" Lorraine University
Tél: +33 (0)3 83 68 42 28
Mail: eric.gelhaye@univ-lorrraine.fr

General aims and state of the art:

Besides their important function in global carbon cycle, the expansion of agro-industrial industry leading to an accumulation of lignocellulosic residues coupled to the need of new source of energy (second generation of biofuels) has led to an increasing interest for wood/litter decaying fungi. Many organisms are able to degrade and use cellulose and hemicelluloses as carbon and energy sources; however microbial wood degradation seems to be restricted to fungi, the most efficient being basidiomycetes. The oxidative processes used by these fungi to degrade lignin generate a myriad of potential toxic molecules depending in particular of the wood composition. In addition, as other microorganisms, these fungi have to adapt with antagonistic interactions more or less specific of their habitat and to changes due to the human activities (environmental pollutants for instance). The recent release of sequencing genomes of various agaricomycetes allows exploring the extraordinary ability of these fungi to cope with these potential toxic compounds. Besides the ability to degrade/modify lignocellulose, we assume that the detoxification pathways found in these plant interacting fungi reflect their local adaptation to their habitat, this latter being mainly due to wood chemical composition (Cornwell and Ackerly, 2010).
The mechanisms and the gene networks involved in this adaptation could be mainly due to extension/contraction of gene families in particular those involved in stress response. Our preliminary data performed on the GSTome of the

wood decaying fungus *Phanerochaete chrysosporium* revealed that duplications have been followed in all investigated cases (15 isoforms) by neo-functionnalisation processes leading to different specificities/activities in the various investigated enzymes Furthermore, we have developed a strategy using fluorescent substrates allowing either detecting GST substrates from complex mixtures as wood extractives or from screening chemical libraries. Using these tools, we have been able to detect specific substrates for different PcGSTs isoforms in beech extractives (Meux et al., 2012). Interestingly, beside the classical genes involved in stress responses, we have highlighted the induction of genes coding for small secreted proteins (SSP) in response to oak molecules. These small proteins do not exhibit any homology with sequences available in the databases. They are predicted to be secreted and contain many cysteines. Similarly the secreted proteome of *Trametes versicolor* revealed the presence of such proteins when the fungus was grown directly on oak compared to a control without wood. The analysis of the secretome of various isolates of *T. versicolor* grown on oak revealed that SSP represent 3% of the secreted proteins. A clear relationship has been observed between wood specificity and secretion of such proteins. SSP are of great interest in pathology and symbiosis research since they could act as signalling molecules in fungi, able to regulate gene expression of the plant host (Hacquard et al., 2012; Plett et al., 2011), however their function is still unknown in wood-degrading fungi.
All these data are consistent with the selection of these expanded detoxification or signalling families due to an adaptation to the degradation of various recalcitrant compounds.
The global aim of the project is to connect the genomic polymorphism of these families and the environmental adaptation of fungi, by functional analyses.

Specific research topic:

The PhD project will be structured around two main points:
1- Our genomic analyses of various strains of *Trametes versicolor*, coupled with the numerous data becoming available among the scientific community for other species, will allow identifying rapidly evolving genes. We will focus on detoxification, antioxidant and signalling systems since they belong to the main mechanisms developed by fungi to cope with toxic molecules released during wood degradation, and oxidative stress.
2- We have recently developed an efficient method to genetically transform *Phanerochaete chrysosporium*. This new result opens important perspectives for functional characterization of proteins of interest. In particular, the functional characterization of SSP will be carried out. Since some SSP genes of *P. chrysosporium* are induced in presence of oak extractives, we can test the interactions of SSP with these wood compounds. A focus will also be done on GST since we have a strong expertise with these proteins at the biochemical level. Despite the described involvement of some GSTs in stress responses, in detoxification of xenobiotic compounds, in cell development via some hormone ligandin properties and in secondary metabolism, their catalytic specificities and precise physiological functions are unknown in most cases. At the cellular level, some GSTs are involved in cell protection during the wood decomposition process, which generates toxic compounds via (i) a catalytic detoxification activity and ii) a ligandin function to sequester and transport toxic molecules. We intend to functionally characterize these proteins by identifying their specificities toward physiological substrates/metabolites.

Novelty and relevance to the research project of the team:

The novelty of the project concerns mainly the development of methods to connect genotype and phenotypes developing functional and physiological approaches. A lot of genomic data are indeed available, however very few *in vivo* characterizations have been performed to date to understand fungal adaptation to their substrate. Moreover, most of the studies concern the extracellular network responsible for biomass degradation. In this project we propose to study the mechanisms by which fungi have evolved to resist to the toxic molecules released during wood degradation.

Potential impact for the scientific discipline and the society:

Plant cell walls are high potential renewable resources for the production of biofuels. Complex raw materials from different origins (dedicated crops, agricultural wastes, silviculture, etc) constitute new potential sources of sugars that can be fermented for production of 2nd generation bioethanol. Another key-point is the valorization of by-products of biorafinery, such as phenolic compounds and lignin. Polyporales are an extraordinary resource to

identify novel enzymes or synergistic factors that contribute to efficient biomass degradation or transformation. In particular, oxidative enzymes are increasingly studied for their key role in the enzymatic mechanisms developed by Basidiomycetes to degrade lignocellulose. Such oxidative enzymes are used to complement commercial enzymatic cocktails originated from the industrial fungal work-horses (Ascomycetes fungi *Aspergillus niger* and *Trichoderma reesei*). The integrative analysis of genomics, transcriptomics and enzymatic functionalities will provide an efficient way to identify the key enzymes involved in plant cell wall deconstruction. It is also expected that this strategy will unravel the synergetic activities of enzymes recruited by the fungi and guide the conception of enzymatic combinations to improve the performance of bioprocesses.
Comparative genomics coupled to protein biochemistry and wood chemistry provide a promising new systems-biology approach to understanding the molecular processes of forest soil fungi that drive the element cycles of the boreal and temperate forest biomes. In this project, we aim at determining how Agaricomycotina have evolved to efficiently decompose and assimilate carbon in forest ecosystems**,** and studying how the environment might have influenced genetic diversity and gene expression. Moreover, deciphering how the chemical composition of wood infers on its susceptibility to biological attack remained a bottleneck for the development of environmental-friendly wood preservation strategies. Apart from these socio-economic interests, the research developed in the PhD project will have strong academic and biotechnological issues.

Available equipment / experimental support / associated research projects:

The specific tasks of this project could be divided into three main tasks:
1. Comparative genomics in order to identify key gene families involved in the adaptation of wood decaying fungi
During the SYMWOOD project funded by ARBRE in collaboration with LERMAB, we were able to resequence 10 strains of *Trametes versicolor* allowing comparative genomic studies at the intraspecies level. The IAM department has and is also involved in the sequencing of numerous genomes available on the JGI Mycocosm website. Bioanalysis (bioinformatics and biostatistics) will be performed in the lab with the support of specialists in particular within the ecogenomic team of our department.
2. Functional analysis of various multigenic families
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 a method to "fish" natural ligands of a protein by affinity chromatography and competition tests using fluorescent probes. This technique has been successfully used to characterize the ligandin property of glutathione transferases (Meux et al., 2013; Mathieu et al., 2012). In case of specific ligand identification, a collaboration with CRM2 (Lorraine University) will allow the resolution of the 3D structure of the complex to give additional information on how it functions. We have also the expertise and the tools to screen and identify putative interactions with other proteins (affinity chromatography, fluorescent tools, microcalorimetry...). Concerning SSP, the same approaches as those descibed for GST could be used. In particuler, putative substrates could be identified by using *in vivo* ligand fishing method after overexpression of Strep-tagged or His-tagged GSTs in fungal cells. The analysis of mutants (overexpressor or RNAi) will also be performed. The recombinant proteins can also be produced in fusion with a reporter protein to follow its binding ability onto wood materials and its ability to move within the hyphal network by microscopic techniques. Finally, the effect of SSP accumulation could be analyzed at the phenotypic level. We will also test whether SSP accumulation modifies the proteome of the fungus by 2D electrophoresis.

Skills that the doctoral fellow will gain during the contract:

Cognitive skills: Cellular biology, Functional ecology, Biochemistry, English language, Production of reports and articles.
Technical skill: Functional genomics, Transcriptomics, Bioanalysis (bioinformatic analysis of genomic), Enzymology

Thesis committee (*Suggested composition)*

Pr Stéphane Vuillemier (Strasbourg), Dr Roland Marmeisse (Lyon), Pr Philip Board (Canberra, Australie), Dr Stéphane Lemaire (Paris).

Five publications of the research group on the topic:

Morel M, Ngadin AA, Droux M, Jacquot JP and Gelhaye E. (2009). The fungal glutathione-*S*-transferase system.Evidence of new classes in the wood-degrading basidiomycete*Phanerochaetechrysosporium.*Cellular and Molecular Life Science Aug 7.

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