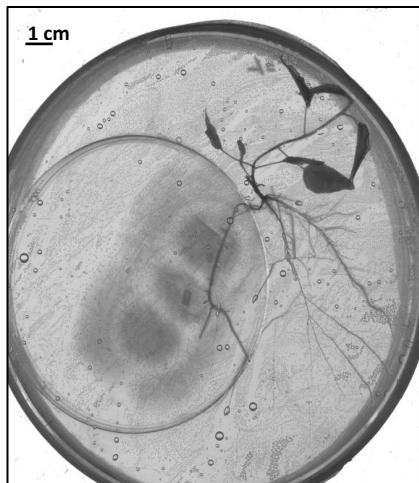




NUTRRESYM



NUtrient signals in forest Trees Regulating Root growth and Ecotmycorrhizae SYMbosis

Principle investigator: Clémence Bonnot, UMR Interactions Arbres/Micro-organismes (IAM)

LabEx partners: Annegret KOHLER (IAM), Marie-Béatrice BOGEAT (EEF), Pascale MAILLARD (EEF), Dominique GERANT (EEF), Francis MARTIN (IAM)

With the collaboration of: Paul Abraham Oak Ridge National Laboratory (USA)

Work packages: WP1 (1.1 and 1.2)

To improve their mineral nutrition, trees form symbiotic associations with mutualistic microorganisms [1]. Symbiotic associations are mutually beneficial interactions in which the microorganism provides the plant with mineral nutrients in exchange for organic carbon. These associations have an energetic cost for the plant such as the modification of roots architecture to explore and adapt to the heterogeneity of nutrients in soil. To maintain an optimal growth, plants have to integrate environmental and metabolic nutritional cues to regulate adequately their symbiotic associations and root architecture [2]. In Legumes, Small-Secreted Peptides (SSP) mediate local and systemic signals regulating nutrient stress responses including symbiotic interactions with nitrogen fixing bacteria as well as lateral roots growth [3,4]. Occurring between 6000 trees species and several fungi of the Basidiomycota and Ascomycota phyla, ectomycorrhizal (ECM) symbioses are pivotal for nutrient cycling in forests [1]. However, little is known regarding the molecular and physiological mechanisms controlling ECM associations [5]. Members of several SSP families were found in the model tree *Populus* [6,7]. To investigate whether and how trees nutrient signals and SSPs control the formation and maintenance of ECM associations, we (i) characterized the effects of nutrient stresses on ECM symbiosis in *Populus X canescens* associated to the ECM fungus *Laccaria bicolor*, (ii) identified the members of SSPs families in *Populus* by genome scan and phylogenetic analysis, (iii) selected candidates peptides looking at their transcriptomic and proteomic response to nutrient signals in polar and ECM interaction between poplar and ECM fungi. We discovered 6 SSPs transcriptionally induced by ECM interactions in poplar capable of increasing significantly the number of ECM roots formed. These peptides are promising candidates for the regulation of ECM in poplar.

- [1] Martin et al. (2016) *Nat Rev Microbiol* 14, [2] Nehls et al. (2016) *Mol. Mycorrhizal Symbiosis* 161–178
- [3] de Bang et al. (2017) *Curr. Opin. Plant Biol.* 39, 31–39 [4] Bisseling et al. (2014) *Science* 346, 300–301 [5] Garcia et al. (2015) *New Phytol.* 208, 79–87 [6] Ghorbani et al. (2015) *J. Exp. Bot.* 66, 5257–5269 [7] Goad et al. (2017) *New Phytol.* 216, 605–616

Context — Mineral nutrients, in natural soils and specifically in forest soils, are scarce and heterogeneously distributed (Attiwill and Adams, 1993). To improve their mineral nutrition trees have to invest energy in the production and functioning of nutrient acquisition organs such as roots and symbiotic organs allowing them to interact and exchange nutrients with symbiotic microorganisms. This is the case of trees that forms symbiotic roots called ectomycorrhiza (ECM) with ectomycorrhizal fungi (ECM). The formation and maintenance of these organs (both roots and ECM) have a high carbon cost for the plant (Hodge et al., 2009; Nehls et al., 2016; Farrar and Jones 2000). Hence, in zones of the soil in which the availability of mineral nutrients is high, the formations of a high number of roots and ECM have deleterious effects on the plant biomass. To maintain an optimal growth and invest their resources efficiently, plants have to integrate the nutritional clues of the environment and metabolism to regulate adequately the formation and functioning of these organs (Nouri et al., 2014; Evans et al., 2001).

In many herbaceous plants, small secreted peptides (SSPs) of several genes families mediate the local and systemic nutritional signals regulating the formation od roots and symbiosis (de Bang et al., 2017; Bisseling and Scheres, 2014; Gutiérrez-Alanís et al., 2017). However, the molecular mechanisms allowing these regulations in trees remain unknown.

Objectives — Members of several SSP families have been identified in different tree species including poplar (Ghorbani et al., 2015; Goad et al., 2017). In order to unveil the mechanisms allowing trees to regulate their root architecture and symbiotic interactions in response to their nutritive environment and status, we propose to verify the role of SPPs in the regulation of ECM symbiosis and root architecture in poplar in response to four nutritive stresses: starvation and excess phosphate (Pi) and starvation and excess nitrates (NO_3^-).

Approaches — To investigate this, (i) we characterised the effects of those four nutrient stresses on the root system of the model poplar *Populus X Canescens* in association to the model ECM fungi *Laccaria bicolor*, (ii) we identified by bio-informatics and phylogenetics the SSP families present in the genome of *Populus X Canescens*, (iii) we used transcriptomics and proteomics to select SSPs candidates for the transduction of nutritive signals regulating the root system architecture and establishment of symbiosis in poplar and finally, (iv) we verified the direct effect of those candidates SSPs on root architecture and ECM formation by *in vitro* treatment of poplar in interaction with *Laccaria bicolor* with these peptides.

Key results —

- (i) In our growth conditions, only the NO_3^- concentration of the medium did affect the root system of poplar. NO_3^- starvation favoured the local formation of ECM. In zones of the root system directly in contact with low- NO_3^- medium, the number of symbiotic organs formed increased as well as the of the size of the exchange surface between the plant and the fungi (Hartig net) in these organs. The formation of a deep Hartig net inside the ECM roots is a strong indicator of their well function (Plett et al., 2014). NO_3^- starvation may favour the functioning of ECM. Nor the Pi neither the NO_3^- stresses did affect poplar root architecture.
- (ii) 21 families of SSPs were identified in poplar. Two of them being known to have members driving nutritive signals in legumes and *Arabidopsis thaliana* (CEP and CLE).
- (iii) Amongst the peptides of the CEP and CLE families, two groups of candidates were selected based on their transcriptional response to NO_3^- and to the interactions of poplar with several ECM fungi (*Laccaria bicolor*, *Amanita muscaria*, *Cenococcum geophilum* et *Pisolithus microcarpus*): the peptides regulated by NO_3^- (Group A) and the peptides regulated by the ECM interaction (Group B). Group A peptides may be involved in regulating symbiosis in response a nutritive stress. Group B peptides may be involved in the regulation of the establishment of the ECM symbiosis or in an-symbiosis auto regulation signal such as those existing in legumes.
- (iv) Treating *in vitro* *Populus X Canescens* in interaction with *Laccaria bicolor* with synthetic peptides of those two groups showed that some of the group B peptides increase the formation of ECM. No effects were observed for the chosen group A peptides.



Main conclusions including key points of discussion — Our results suggest that poplar regulates its interaction of the ECM fungi *Laccaria bicolor* en response to NO_3^- but not Pi . Pi starvation and excess do not modify the formation or the structure ECM roots. Though, we cannot exclude that Pi might affect the functioning of ECM roots or that its effects is dependant on the fungi specie in symbiotic interaction with poplar. Recording the exchange of C, N and P between the tree and the fungi is needed to fully understand the final effects of those stresses on the ECM symbiosis.

The majority of the SSP families identified in poplar are orthologous to the SSP families already known in herbaceous species. This is the case of the CEP and CLE families which members are known to drive the nutrient and hydric stress signals in legumes and *Arabidopsis thaliana*.

Group A peptides are regulated by NO_3^- and as such might be responsible for the NO_3^- starvation and excess signals repressing/inducing the establishment and/or maintenance of symbiotic interactions. There are good candidates for the signal leading to the observed induction of ECM by NO_3^- starvation. The fact that none of the candidates peptide of group A had any effect on the formation of ECM interactions in our *in vitro* treatment do not allow us to conclude that none of them has any role in the regulation of ECM symbiosis by NO_3^- . Indeed in our conditions, NO_3^- was present in the medium and may have masked the ability of some of these SSPs to repress the formation of ECM. Therefore they should be tested again but on low- NO_3^- medium. Moreover, more SSPs than those tested in Group A, were transcriptionally regulated by NO_3^- stresses and should be synthesised and tested.

Contrary to the group A peptides, some of the group B peptides (induced by ECM symbiosis) were found to stimulate the formation of ECM. This suggests their involvement in the establishment of the symbiotic interaction or in the formation of the ECM organ itself. Their effects on the physiology of the symbiosis as well as on the tree-fungi interaction must be explored. This may be done by using transgenesis to produce poplar Knock Out mutants or over expressers of the genes encoding those peptides. *Laccaria bicolor* may be transformed; hence the production of *Laccaria* strains over-expressing these SSPs of interest may be considered.

Perspectives — Our results are preliminary in the comprehension of the nutrient signal regulating the symbiosis and root architecture of trees but are extremely positives. They show that SSPs do have a role in the establishment of symbiotic interactions in trees. Some are clearly able to increase ECM interactions. Their exact roles in the establishment of symbiotic interactions in tree must be explored. The level at which they act (systemic or local regulation) and context (establishment of the symbiosis or manipulation of another pathway by the fungi) are yet to unravel.

The discovery of those peptides suggests that others may regulate symbiosis in response to nutritive signals. The identification of SSPs induced and repressed by NO_3^- grounds this hypothesis. Their effects on the ECM symbiosis in low- NO_3^- remain to be tested.

This open a whole world of possibilities to study signals in trees and their abilities to respond and adapt to their environment which should be explored in more species to verify whether the same signalling pathways are found and if their outcome can be predicted in different species.

Valorization —

Publication: Villalobos Solis, M. I., Poudel, S., Bonnot, C., Shrestha, H. K., Hettich, R. L., Veneault-Fourrey, C., ... & Abraham, P. E. (2020). A viable new strategy for the discovery of peptide proteolytic cleavage products in plant-microbe interactions. *Molecular Plant-Microbe Interactions*, 33(10), 1177-1188.