Our main goal is to optimise the production and to test the efficiency and the innocuity of biocontrol products (BCPs) and of a bioamendment, both produced via solid-state fermentation of agricultural by-products. Up to now, few solutions exist to limit the use of pesticides and of copper sulfate in viticulture though the European Union wants to decrease the concentration of $CuSO_4$ used per ha and per year. Thus, it seems of huge importance to favor the production of non-harmful BCPs and of a bioamendment, valorising agricultural by-products in the context of a circular, local and autonomous economical strategy.Researches based on microbial interactions to define new biocontrol agents in agriculture are still poorly developped when compared to those about synthesised pesticids. Identifying antagonistic fungi as biocontrol agents producing metabolites with antifungal potential is thus of huge importance. Genus *Trichoderma* is considered as a powerful antimicrobial agent which can produce lytic enzymes (such as cellulase, amylases and lipases), antifungal metabolites and spores (ultrastructure resistant to dessication and heat). By limiting the impact of various inputs (pesticids, $CuSO_4$, mineral fertilizers) which lead to a decrease in soil quality and can be considered as a threat for biodiversity in agrosystems and for human health, by favoring agricultural by-products recycling, this project perfectly matches the scientific purposes of environmental transition.

We will aim at i) developping a BCP cocktail with a strong antifungal activity, using the conditions of culture of *Trichoderma* under solid-state fermentation that have been already described by the PhD thesis of R. Hamrouni and testing this cocktail on main *Vitis* phytopathogens (*Botrytis cinerea, Plasmopara viticola, Erysiphe necator*), (ii) producing a new bioamendment based on forest litter (as inoculum) under anaerobic conditions, the potential of this bioamendment has been proven empirically though the processes underlying its effects (enhancing soil qualities ans plant growth) need to be deciphered. , (iii) testing the innocuity of both the bioamendment and BCPs using genotoxicology on human cells (both somatic and germinal), (iv) promoting the changes of agricultural practices by farmers using psychosocial strategies based on a partnership between winegrowers and researchers together with associative and professional partners (ODG Côtes de Provence, Association Vignerons de la Sainte Victoire, Association Terre et Humanisme, Chambre régionale de l'agriculture) (v) recycling/ valorising agricultural by-products for a local production by the farmers of products of fermentation under the context of a circular and autonomous economy.
This innovative study relies on transdisciplinarity, combining environmental sciences *sensu lato* (microbial biotechnology, chemistry, genotoxicology, plant ecophysiology, agronomy and soil science) and social sciences (link between farmers and scientists, language analyses, landscape analyses), which is mandatory for the success of such project.

This work is organised in 6 main tasks :

Task 1/ Production of a cocktail of BCPs and in vitro test on phytopathogens *Botrytis cynerea, Erysiphe necator* Schwein et *Plasmopara viticola.* Coordinator N. Dupuy. Contributors: PhD student, A.M. Farnet, J. Molinet, L. Foli, M. Martinez, J.L. Boudenne. Metabolites with antifungal properties will be produced under solid state fermentation (SSF), mainly 6 pentyl-alpha-pyrone, 6PP), enzymes (lipases, amylases et cellulases) and also conidies using different strains of *Trichoderma asperellum* (TV104, QT22046 et TF1 to test intraspecific variation of virulence) and the antifungal activities of these metabolites will be analysed towards the principal phytopathogens of *Vitis* (*Botrytis*, oïdium and mildiew). This first task will take advantage of the previous work of R. Hamrouni (PhD thesis, Hamrouni et al., 2019 a, b, c , d) who has already optimised SSF conditions for the production of such compounds. From SSF substrate, an aqueous extract including BCPs will be fractionated (steric exclusion, polarity) and each fraction will be analysed by UV-Vis and fluorescence-3D (LCE, JL Boudenne). I*n vitro* assays testing the effect of these different fractions on phytopathogens will be performed according to Gabaston et al., (2017): disks of *Vitis* leaves will be soaked with each fraction and then will be subjected to a spray of a suspension of conidies of phytopathogens (IMBE, A.M. Farnet). The different varieties of *Vitis* commonly planted in Provence Alpes Côte d'Azur (PACA) region will be selected to test the variability of responses depending of the grape variety. An experimental design will include 4 factors: strains of *T.asperellum* (TV104, QT22046 et TF1), pathogens (*Botrytis cinerea, Erysiphe necator* Schwein et *Plasmopara viticola)*, spore concentrations and grape variety to identify the effects of factors on pathogen sporulation and growth. Previous studies have given proofs of antibiotic effects of various metabolites produced by *Trichoderma* (for instance 6PP) on numerous phytopathogens and also a stimulating effect of the mechanisms of plant defense (production of peroxidases and phenoloxidases).(El-Hasan A. and Buchenauer, H., 2009, Vinale et al., 2009). Moreover, the stability of 6PP (photolyse and hydrolyse) will be assessed under laboratory experiments (artificial solar light exposure) and in mesocosms (task 4) (analyses via LC-MS/MS). The toxicity of the compounds potentially produced will be tested in task 3.

Task 2/ Characterisation of physico-chemical (quality and quantity of organic matter, total C and N …) and microbial (ratio between saprophytrophic and pathogens, microbial activities, active microbial biomass) properties of the bioamendment. Coordinator P. Christen. Contributors: *PhD student*, A.M. Farnet, L. Foli, J.-L. Boudenne, Making bioamendment for forest litter is a simple technique – that farmers can perform- and innovating under temperate climates. It is based on an anaerobic fermentation *sensu stricto*. Its effect on soil fertility and on crop production have been demonstrated (Singh et al. 2011) such as improving physico-chemical properties of soils from agrosystems (Valarini et al. 2002, Condor Golec et al. 2007) or technosols (Zornoza et al. 2017). Bioamendment inputs could also mitigate the effect of abiotic stresses such as drought (Grover et al. 2011). To prepare such bioamendment, a few quantity of forest litter is needed (here *Quercus ilex*, a representative species of the Mediterranean context, rich in nutrients compared to coniferous species) and is used as inoculum in an admixture of agricultural by-products as bran (rich in cellulose), green wastes from *Vitis* cut, treacle and whey (Dantinne et Reyes 2018). Fermentation is conducted at room temperature (around 20 °C) for one month under anaerobic conditions. Triplicates of 10kg wet weight will be performed. Before spreading onto soil, the bioamendment is activated by mixing 3 kg of the product of fermentation with 3 kg of treacle and 3kg of whey qsp 60L of water. This admixture is then fermented at room temperature under anaerobic conditions for 6 days (Dantinne et Reyes, 2018). Genotoxicity tests will be performed on aqueous extracts of this admixture which is supposed to be spread onto agricultural soils (Task 3). Characterisation of the fermentation products will be realised i) at the end of the first SSF (5 composite samples per replicate ; 10 samplings per composite sample). Chemical properties of the fermentation product will be described *via* elementary analyses (C and N), solid-state NMR of ^{13}C and Infrared spectroscopy (qualification of organic matter), pH, conductivity, cations and anions, microbial respiration and biomass (substrate-induced respiration), ii) on the activated product of fermentation (i.e. bioamendment sensu stricto) using the previously mentionned methods.

Task 3/ Evaluation of genotoxicity of fermentation products : the bioamendment and BCPs Coordinator T. Orsière, Contributors: J. Perrin, V. Tassistro, JL Boudenne, *Post Doc.*

The bioamendments and BCPs will be produced as liquids and will be sprayed onto soil and leaves which may lead to human exposure via inhalation (winegrowers and inhabitants near vineyards). Moreover, these products may be degraded by UV or water and consequently form other metabolites (identify in Task 1). Consequently, the toxicity of these by-products and of the different aqueous fractions obtained in Task 1 have to be determined (genotoxicology). The more deleterious effects on human health are those linked to mutations on genes or chromosoms. These effects are known to be tenuous and to initiate cancers or to impact fertility when somatic (mainly in leaver) or germinal cells are exposed. We will thus test aqueous solutions of either bioamendment of BCP using a combination of various tests (PIG-A, Comet assays, micronuclei test) that inform about primary lesions of DNA and genetic mutations. These effects will be analysed on cell lines BEAS-2B (human bronchial epithelium) and on ovarian and testicular cell lines as well as on human spermatozoids. 6PP is used as coco aroma and is clasified as 'Generally Considered As Safe' (Food and Drug administration), thus its genotoxicity is unlikely. However its toxicity will be evaluated alone or in combination with the other molecules of the aqueous fractions of BCPs.

Task 4/ Testing the effects of both the BCPs cocktail and the bioamendment on the plant fitness and the physico-chemical (C, N, cations, density, water holding capacity, organic matter quality by NMR and Infra red spectroscopy) and biological (microorganisms, meso and macrofauna) properties of soils from vineyards. Coordinator Y. Capowiez. Contributors: *PhD student*, I. Laffont-Schwob, C. Pelosi, N. Dupuy, J. Molinet, A.M. Farnet Da Silva, P. Christen, P. Prudent, J.L. Boudenne, H. Folzer.

Inputs of both the BCPs cocktail and the bioamendment onto 15L mesocosms of postagricultural soils (less impacted by various inputs) with grape vines at the same stage of growth, will be performed for 2 years. Treatments (5 replicates) are the following : (1) control without treatment, (2) BCPs (200 mg/l equ 6PP), (3) $CuSO_4$ (at the authorised concentration),(4) bioamendment, (5) BCPs (200 mg/l equ 6PP) + $CuSO_4$, (6) bioamendment + BCPs (200 mg/l equ 6PP) (7) bioamendment (200 mg/l equ 6PP) + $CuSO_4$, (8) bioamendment + BCPs (200 mg/l equ 6PP) + $CuSO_4$ to test potential synergistic effects of bioamendment + BCPs and to take into account the fact that winegrowers will first use both CuSO4 and BCPs. Another factor will be tested : the presence of macrofauna (earthworm *Aporrectodea caliginosa*) and mesofauna (= *Enchytraeus albidus*) to (i) test potential ecotoxicological effects of the treatments on soils fauna and (ii) detect the synergistic effects of fauna with treatments via bioturbation and degradation of the bioamendment by soil fauna (INRAE). BCPs concentrations will be established according to the litterature (Poole et al., 1999, El Hasan, 2008). Bioamendment will be applied at a concentration of 3% (dilution of the activated bioamendment) using 300 mL/m^2 (Dantinne et Reyes, 2018), once a month from march to may and twice a month from july to september. **80 mesocosms will be set up :** 5 replicates x 8 treatments x with or without meso/macrofauna. Plant traits (number of stems, twigs etc…) will be followed overtime as well as phytometabolites using a non-destructive approach (Multiplex, Force A) and the degree of phytopathogen growth will be estimated via spots of fungal growth on leaves (Schnee et al, 2013). Aerial plant biomass as well as root biomass (winrhizo) and chemical analyses of plant organs (phytometabolites, NMR and Infra red spectroscopy, copper in plant organs) will be performed at the end of the experiment (IMBE, LPED, LCE). The survival rate of meso and macrofauna will be assessed (the state of development will be taken into account). Physico-chemical (pH H_2O, pH KCl, C, N, cations by ICP-MS, density, water holding capacity, organic matter quality by NMR and Infra red spectroscopy), biological (meso et macrofauna i.e . mainly enchytreids and earthworms) and microbial (microbial respiration and biomass by substrate-induced respiration, lignocellulolytic activities, proteases, ureases, phosphatases, catabolic diversity) properties will be analysed in mesocosm soils. Copper in soil (chemical speciation : pseudo-total fractions, phyto-disponibility and water lixiviates) will also be measured

Task 5 / Parternship between winegrowers/researchers for new practices favoring environment in viticulture : role of cultural identity and tradition, social rules and sustainable practices in agriculture in South-Eastern France. Co coordinators : Raquel Bertoldo & Paul Minvielle Larrousse ; Contributors: Bouchra Zouhri ; *PhD student*, *deux M2*, A.M. Farnet Da Silva, I. Laffont-Schwob. Vineyard is emblematic of a culture legacy, tradition and sustains regional economy. This economic and social activity has built a professional identity overtime and generation and also has created links between institutions, professionals and associative lives. This task will aim at understanding and deciphering social and territorial practices in viticulture to establish partnership between all the actors of the project and the winegrowers to favor acceptance of new practices in viticulture to protect agrosystems and their environment (biocontrol products, bioamendment, irrigation, management of land). Winegrowers are already involved in the definition of new practices via local Technical Comitees for instance in the context of « Plan Ecophyto II » sustained by the French Government (ministère de l'agriculture), of the promotion of a viticulture with « High Environmental value ». It is thus of huge importance to establish a partnership with winegrowers and researchers to make the « research-action » strategy valuable. Studies about changing social practices for a sustainable environment have emerged in the PACA region. For instance, hunters have been involved in strategies for ecosystem preservation (Guimelli, 1989). However, the possibility for wine growers to use alternative options to replace pesticides is scarce and few farmers have taken taking these first steps : those who have been formed and those who have chosen this type of practices from the begining of their activities (Zouhri et al., 2016). Our goal is to understand to which extent farmers will agree to use these new solutions of phytopathogen biocontrol and under which conditions. Three studies will be set up to address this question.

Study 1. Qualitative study about the winegrowers' group membership vs the acceptance of new solutions of biocontrol. Analyses of the social identities based on the choice of certain practices and how these rationales lead to determine that biocontrol solution acceptance is disruptive or not when considering existing social identities.

Study 2. Qualitative study about relationship between farmers and scientists and how this relationship can be considered as threatening their « savoir-faire » (Block, Jensen & Kaltoft, 2008). More precisely what is the permeability of winegrowers' professional practices to new options from « Science » that could enlarge their solutions.

Study 3. Qualitative study about existing practices and partnership for sustainable agriculture : action of local TEchnical Comitees, parternship with Institutions (« instituts techniques », chambres d'agriculture, INAO, Institut National de la Recherche et de la Qualité).

Task 6 / Interactions between disciplines & managment of transversality Co coordinators I. Laffont-Schwob, & A.M. Farnet Da Silva, Contributors: the entire consortium. Management of the projet (workshops with all the actors, field work in experimental sites….), congress to present the consortium results including, scientists, winegrowners, institutions, students, public). Building an open access database about the chemical and biological data from this study (helpful to develop projects with students).