

Impact Objectives

- Develop innovative tools adapted to analyse fungal-environment interactions
- Track fungicide resistance of plant pathogens using long-read sequencing

Mycological evolution

Molecular biologist **Dr Semcheddine Cherrad** discusses the novel tools the **Conidia Coniphy** team has developed to better understand the interactions between fungi and the environment



Can you share a little about your research background?

I have always been fascinated by infinitely small microorganisms like bacteria, viruses and all kinds of cells. Curiosity to know more and understand their role in nature and our lives, which can be beneficial or threatening to human health, drove me to pursue a career in life sciences. During my thesis, I had the opportunity to work in the research and development centre of Bayer Crop Science in Lyon in collaboration with Conidia Coniphy, discovering proteomics and molecular biology. After 15 years working at Conidia Coniphy, molecular biology remains one of my favourite fields and I am continuously training to update my expertise regarding new technologies such as qPCR (quantitative polymerase chain reaction), ddPCR (Droplet Digital polymerase chain reaction), pyrosequencing, NGS (next-generation sequencing) and ONT (Oxford Nanopore Technologies) sequencing.

How are you developing innovative methods at CONIDIA CONIPHY lab?

Molecular biology in our lab is used to detect and characterise microorganisms in different applications like cosmetics, air quality, soils and phytopathology. We have historical experience in the interactions between fungi and the environment. These interactions encompass the beneficial role

of fungi in food (such as cheese processing and interesting secondary metabolites) and the impact and risk in food spoilage or plant health. Compared to bacteria, less knowledge and methods exist in mycology, which drove us to develop innovative tools adapted to analyse fungal interaction. These include the development of image analysis software to count spores and monitor the sensitivity of some fungi to fungicides, the development and management of fungal strain databases, the development of a medium to identify fungi and a patented method for the early detection of a plant pathogen in grapevines.

What knowledge gaps are you filling with your studies?

Just one analysis with ONT sequencing replaces 10 different molecular methods in the case of the *Plasmopara viticola* model and gives more reliable information. ONT long-read sequencing appears to be a promising method for assessing fungicide resistance of pathogens because we can analyse multiple gene targets, meaning detecting resistance to many fungicides with different modes of action. Furthermore, an effective fungicide-resistance surveillance system is essential to detect fungicide resistance quickly before they spread, leading to preserving fungicide effectiveness and crop protection.

What have you learnt about your research into the grapevine disease downy mildew *P. viticola*?

Resistance to fungicide observed in the field in France with monitoring by bioassay was confirmed with ONT sequencing analysis. The proof of concept of this tool was validated on the gene target (cytochrome b) of complex III inhibitors fungicide. In a second step, we analysed targets of other fungicides such as zoxamide and oxathiapiprolin, and we provided an earlier detection of resistance to oxathiapiprolin in France. Resistance to zoxamide was not detected in France. Collaborative work with our partner in the Tentamus group, Epilogic GmbH, scaled up the method at the European level with a Europe Mutation MAP affecting *P. viticola* and conferring resistance to fungicides.

Can you talk about what the ultimate impact of this work is?

This research provides a new method to alert and measure resistance against fungicides used in the field. In global agriculture awareness of the use of fungicides, this tool can provide data to better manage treatment respecting the environment and preserve the effectiveness of a molecule in the field as much as possible. We will be extending methods and analysing pipeline to track fungicide resistance in other plant pathogens, including *Septoria tritici* blotch in wheat crops and grapevine powdery mildew caused by *Erysiphe necator*. ►

Tackling fungicide resistance

Innovations by a dedicated research team at Conidia Coniphy will assist in the earlier detection and monitoring of fungicide resistance

Dr Semcheddine Cherrad, Research and Development Manager at Conidia Coniphy, describes how if plant pathogens can be detected as early as possible, action can be taken to mitigate the spread of disease. In addition to this, as pathogens can develop resistance to fungicides, monitoring fungicide resistance is key to ensuring effective disease control measures. Innovative research incorporating

technological advances in detection and monitoring is advancing plant disease management.

R&D ADVANCING KNOWLEDGE Cherrad's R&D and innovation studies at Conidia Coniphy is advancing microbiological assessment and contamination control. 'We have been working on the detection of plant

pathogens and monitoring of fungicide resistance for over 15 years,' he explains. 'Our team combines expertise in classical microbiological tests called bioassay with molecular analysis, and overcomes challenges by adopting new technologies and processes.' The team is utilising new generation sequencing (NGS) with long-read sequencing to generate huge datasets on DNA molecules of genes coding for

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proteins targeted by fungicides. 'With long-read sequencing, we can sequence all the fungicide target gene sequences and detect multiple mutations along the same sequence giving information on multiple resistance,' highlights Cherrad.

INNOVATIVE TOOLS

One innovation developed by the Conidia Coniphy team is a tool for mould identification called ID-Fungi Plates (IDFP), building on the Maldi-ToF technology. IDFP simplifies and accelerates the identification of fungal pathogens and is also user friendly. Another tool created by the researchers is FUNGI MESURE, which is a tool that can quantify fungal spores in samples, providing an accurate overview of fungal measures and facilitating lab routine tasks of methods with fungi. They have also developed a database called FUNGI DNA that supports the identification and study of fungal strains through DNA analysis by providing a comprehensive collection of fungal DNA sequences.

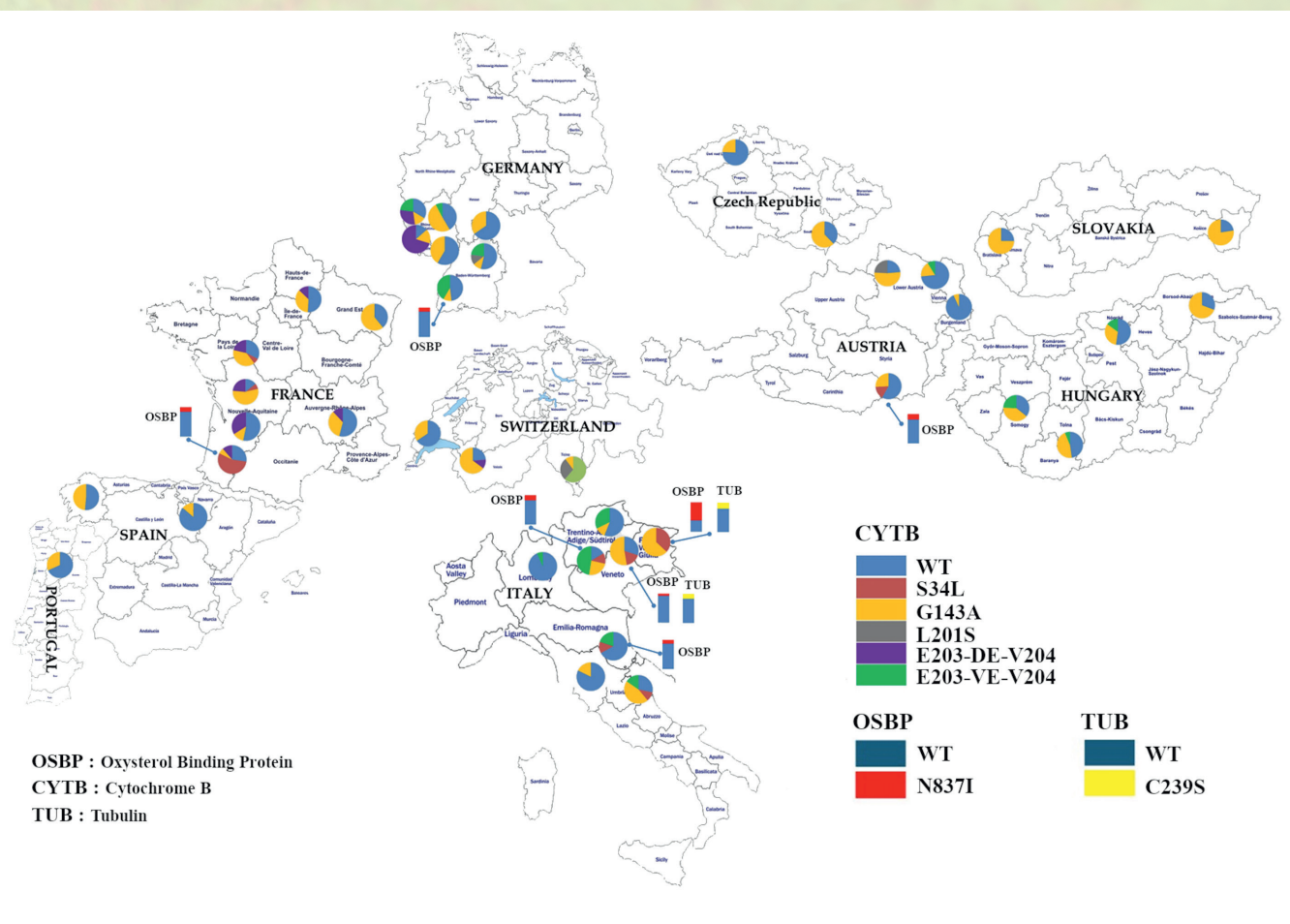
The researchers developed a ready-to-use molecular detection kit for tracking a pathogen called *E. necator* in grapevine. This fungal pathogen causes grapevine powdery mildew, threatening vineyards on a global scale. The molecular detection kit can detect the pathogen at low concentrations and delivers results in a short timeframe. As it is ready to use, it can be used in different settings. This offers the ability to diagnose and manage powdery mildew outbreaks quickly and effectively. 'This was the first kit to be launched in a range of other kits (idetect) to detect plant pathogens and resistance to fungicides,' Cherrad outlines. 'Indeed,

idetect ready-to-use kits facilitate the detection of a range of other plant diseases, including downy mildew of grapevine and diseases affecting cereals.'

TRACKING RESISTANCE

P. viticola is another significant fungal pathogen that affects grapevines, causing downy mildew. In one project, Cherrad and the team investigated the genetic diversity and resistance mechanisms of this pathogen, with a focus on complex III inhibitors, which are widely used fungicides. In French vineyards, *P. viticola* has developed resistance against a group of these fungicides, called Quinone outside Inhibitors (QoI). This is linked to cytochrome b variants. The team collected samples of *P. viticola* from vineyards and used sequencing technologies to obtain a comprehensive view of the cytochrome b gene. 'They then analysed the sequencing data using bioinformatics, which enabled the identification of genetic variants and mutations in the cytochrome b gene,' explains Cherrad. 'The team found significant genetic diversity within the cytochrome b gene of *P. viticola* populations and identified mutations linked to resistance to Complex III inhibitors fungicides,' he confirms. He hopes these findings can inform disease management in vineyards.

Looking ahead, the team will expand their findings and methods to track fungicide resistance in other plant pathogens. They also plan to create a Conidia Coniphy digital lab dealing with new challenges in the prediction of fungicide resistance evolution by exploiting data learning and artificial intelligence serving digital farming. ●



Alleles conferring resistance to complex III inhibitors, zoxamide and oxathiapiprolin in *P. viticola* populations

Project Insights

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TEAM MEMBERS

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BIO

Dr Semcheddine Cherrad has overseen R&D projects in Conidia Coniphy since 2014. He was also involved in applied research projects in the detection of SARS-CoV-2 in air and wastewater, bacteria and enzyme-degrading pollutants in soil, and biocontrol application in plant pathology.



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