



A growing curiosity

Plant pathologist **Professor Louis Bernier** provides an overview of the graduate research centre he co-directs and discusses the ways in which new technologies have revolutionised his field

Could you begin by offering an introduction to the Centre for Forest Research (CFR) and your role within it?

CFR is a multi-university research centre – funded by the Government of Quebec – that trains over 400 master's, doctoral and postdoctoral students. It is the largest forest biology and ecology research group in Canada and one of the most important worldwide. I have been co-directing the Centre with Pierre Drapeau, an ecologist at the University of Quebec in Montreal (UQAM), since 2010.

What are CFR's guiding mission and vision?

CFR's scientific mission is to better understand the functioning and dynamics of forest ecosystems, the interactions between their components and their distinctive biological features, within a perspective that reconciles conservation of biodiversity with increasing basic commercial productivity. Studies conducted by CFR researchers span several spatiotemporal scales, from molecular events to ecosystem processes.

The principal social mission of CFR is to produce highly qualified personnel and contribute actively to the transfer of knowledge to the scientific community, partners involved in the management or protection of forests, and the general public.

Can you summarise your overarching research interests, and explain how you came to develop an interest in the study of tree-fungus interactions?

I have been interested in interactions between trees and other living organisms since high

school. One of my uncles, J André Fortin, was a biologist studying beneficial tree-fungus symbioses known as mycorrhizae, and this also influenced my decision to pursue a scientific career in forest biology. For my undergraduate thesis, I carried out an experimental study of the protective role of ectomycorrhizal fungi against root pathogens. Although results were inconclusive, I was hooked. However, I realised that I was more interested in the bad guys – pathogens – than the good ones – symbionts! Since then, I have been trying to understand how fungal pathogens invade and kill trees, using Dutch elm disease (DED) as my model system.

You use a variety of experimental approaches to conduct your investigations, spanning phytopathology, molecular biology and genomics. Could you provide some insight into your methods?

When studying a biological organism, it is important to link its genotype – genetic makeup – to its phenotype – observable traits. To this end, several complementary experimental approaches need to be used.

In the case of *Ophiostoma novo-ulmi*, the fungus behind DED whose genome was recently sequenced, we now use genome-wide approaches to identify large sets of genes that are expressed under specific conditions – for example, during filamentous growth. We then use standard molecular biology techniques to focus on one or a few genes that we are particularly interested in. In addition, we carry out sexual crosses of *O. novo-ulmi* strains in the laboratory to study the genetic inheritance of traits of interest, and to validate molecular data. Finally, we conduct various morphological and physiological tests for attaching a

discernible phenotype to a specific gene or gene combination. In the case of pathogenicity, we carry out inoculations on elm saplings and apples, since the latter are a convenient and inexpensive substitute for elms in our research.

In what ways is your field changing in response to the rapid development of molecular biology and -omics technologies?

In the past, genetic analyses of fungal pathogenicity were mostly restricted to the species in which sexual reproduction could be obtained in the laboratory. Analyses of complex traits such as pathogenicity also meant that one had to plan and perform complex programmes of sexual crosses. While I believe it is still important to address traditional Mendelian genetics where possible, the rapid development of molecular biology and -omics technologies has sparked a revolution in the way we study biological organisms and the interactions that occur among them.

We can now study the molecular bases of pathogenicity of virtually any fungal species, whether it undergoes sexual reproduction or not. We are also able to directly manipulate genes of interest and establish their contribution, whereas traditional genetics relied on associating a distinctive phenotypic trait with a genetic locus – the position of a genetic sequence on a given chromosome. -Omics technologies also provide the opportunity to perform experiments in which we can measure the expression of all the genes in a single genome. Finally, we now have access to large – and ever growing – databases which facilitate comparisons among organisms and sometimes lead us to formulate hypotheses we would not have otherwise developed.



Understanding **tree-fungus** interactions

A suite of studies undertaken at **Laval University** in Quebec, Canada and collaborating institutes aims to shed some light on *Ophiostoma novo-ulmi*, the fungal pathogen responsible for the devastating Dutch elm disease

SINCE THE FIRST half of the 20th Century, forests across Europe and North America have been battling Dutch elm disease (DED). A serious vascular wilt disease, DED is caused by the invasion of fungal pathogens, most commonly *Ophiostoma novo-ulmi*.

Infection typically manifests itself first in the shrivelling of leaves and perishing of branches, and then spreads quickly throughout the rest of the tree. The disease dramatically shortens the life expectancy of elms, which sometimes die as quickly as one year after contraction. Aided by the elm bark beetle, which rapidly transmits DED from tree to tree, outbreaks have the potential to wipe out elm populations in particular areas, severely impacting upon the biodiversity and economy of a local area.

OPHIOSTOMA: AN IDEAL MODEL

Professor Louis Bernier, co-Director of the Government of the Quebec-funded Centre for Forest Research (CFR), has dedicated much of his career to the study of tree-fungus interactions. Now based at the Laval University in Quebec, Canada, Bernier first came into contact with *O. novo-ulmi* while completing his graduate training, and the pathogen has remained a central focus of his research ever since.

There are a number of reasons for this enduring interest. As well as his desire to learn more about DED and develop solutions for its control, Bernier believes that *Ophiostoma* species provide an ideal model for the training of graduate students with an interest in fungal tree pathogens. "Furthermore, DED fungi are easy to grow under laboratory conditions, are genetically tractable and have life cycles which involve close interactions with trees and bark beetles," he explains.

Thanks to a range of grants from the Natural Sciences and Engineering Research Council of Canada, Bernier has been able to set up a research group dedicated to the investigation of various biological and genetic aspects of *O. novo-ulmi*. Specific research areas include the organisation and plasticity of its genome, the features that lead to its pathogenicity, as well as the fungus's ability to switch between yeast and mycelium growth forms, for which a combination of genetic, molecular and genomic approaches are employed.

ANALYSING O. NOVO-ULMI'S GENOME

A major strand of work was recently completed in partnership with McGill University and Genome Quebec Innovation Centre related to the sequencing and assembly of the *O. novo-ulmi*

genome. Now that the sequencing phase has been completed, the researchers are working on the annotation of the genome.

Non-coding and coding portions have been separated by the scientists, before using public genomic databases and controlled experiments in an attempt to attach significance – for example, picking out the genes that cause pathogenicity – to the coding segments. Bernier and his team are looking for genes known to modulate pathogenicity in other species and assessing whether they act in a similar way in *O. novo-ulmi*, as well as genes unique to this fungus. In order to strengthen the case for a particular gene being behind *O. novo-ulmi*'s pathogenic behaviour, mutants with reduced virulence are also being analysed for genes that have been altered.

EXPLORING YEAST-MYCELIUM DIMORPHISM

Alongside their genomic research, the group is also exploring the molecular basis of *O. novo-ulmi*'s yeast-mycelium dimorphism – that is, its ability to simultaneously grow as a yeast and as mycelium and switch between the two forms. "Our current hypothesis is that this dimorphism is essential for pathogenicity and overall fitness," Bernier elucidates. Several different approaches are being employed to test this theory. In one experiment, the researchers have manipulated

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the fungus under a range of different conditions in order to pinpoint the genes that seem to be involved in its transition between the two states of growth. Next, they plan to take these genes and alter them in order to see if the characteristics of mutated versions vary from that of wild types.

Bernier is also using genomic techniques in this area of investigation. Firstly, the recently sequenced *O. novo-ulmi* genome was analysed for genes similar to those proven to play a significant role in yeast-mycelium dimorphism in other species. In addition, the researchers are carrying out genome-wide transcript profiling experiments in an attempt to ascertain simultaneously the genes that are expressed – or repressed – at each stage of the fungus's life cycle. Ribonucleic acid (RNA) sequencing is being used to pick out the genes that appear to be particularly active during yeast and filamentous growth, respectively. After these genes have been identified, the team can pinpoint them and conduct further tests.

OTHER RESEARCH INTERESTS

While DED is at the heart of much of their research, it is by no means the only line of enquiry being pursued by Bernier and his colleagues. Another project focuses on Septoria canker, a pathological condition caused by the *Mycosphaerella populi* fungus that affects poplars. *M. populi* does not pose much of a problem to poplars native to North America, but has damaged hybrid stocks to the point where resistance to Septoria canker is a key consideration for tree breeders when looking for new clones and selections. Within this section of work, the researchers have assessed the genetic diversity of the fungus among populations found in North America.

The group also took part in a collaborative venture based around sapstain. Sapstain – otherwise known as bluestain – refers to the saprophytic growth of melanin-producing fungi, which leads to the appearance of blue streaks in affected wood. The condition can have a particularly negative effect on the wood

products industry, since sapstained wood is of little commercial value. For this project, Bernier and his colleagues were involved in ascertaining the main fungal culprits of sapstain in Canadian coniferous species, as well as helping to test a biological control method.

THE IMPORTANCE OF WORKING TOGETHER

Collaboration in this research is crucial to provide the highest quality training for graduate students, as well as ensure that the limited funding shared by the forest pathology research community goes as far as possible. Bernier cites a long-term partnership with Dr Will Hintz, a fungal geneticist at the University of Victoria in British Columbia, as fundamental to his work on *O. novo-ulmi*. In addition, he has regularly teamed up with Dr Colette Breuil of the University of British Columbia for his sapstain research and Richard C Hamelin of the Canadian Forest Service in Quebec City for his studies into *M. populi*.

An example of a particularly fruitful collaboration is Bernier's work with Drs Philippe Silar and Fabienne Malagnac of Paris Diderot University. During the 2014-15 academic year he will be joining them in their laboratory to learn about their methods for the study of the filamentous fungus *Podospora anserina*, with the hope of being able to apply these to his own work.

SHARING FINDINGS TO DATE

Bernier showcased his group's research on *O. novo-ulmi* at the International Elm Conference in Florence, Italy in October 2013, with the work presented at this event set to feature in a 2014 edition of iForest. In addition, he plans to attend the 10th International Mycological Congress in Bangkok, Thailand, and give one of his graduate students the opportunity to present the latest findings of their studies. Finally, Bernier has been invited to discuss his team's work at the American Phytopathological Society's meeting in 2014.

Yeast like spores of *Ophiostoma novo-ulmi* © Mirella Aoun



INTELLIGENCE

ROLE OF YEAST-MYCELIUM TRANSITION IN THE PATHOGENICITY OF THE DUTCH ELM DISEASE FUNGUS *OPHIOSTOMA NOVO-ULMI*

OBJECTIVES

To dissect the complex system controlling and modulating parasitic fitness in the highly aggressive Dutch elm disease fungus *Ophiostoma novo-ulmi*. Current projects focus on the identification of pathogenicity genes and the characterisation of regulatory pathways controlling yeast-mycelium dimorphism.

KEY COLLABORATORS

Dr Roger C Lévesque, Laval University, Canada

Dr Ken Dewar, McGill University, Canada

Dr Colette Breuil, University of British Columbia, Canada

Dr Will Hintz, University of Victoria, Canada

Dr Richard C Hamelin, Canadian Forest Service, Canada

Dr Philippe Silar; Dr Fabienne Malagnac, Paris Diderot University, France

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LOUIS BERNIER completed his Master's and PhD degrees studying the Dutch elm disease fungi at the University of Toronto. After postdoctoral work on insect pathogenic fungi at the University of Bath (UK), he joined Laval University where he is currently Professor of Forest Pathology and co-Director of the multi-university Centre for Forest Research. The Dutch elm disease pathogens remain his favourite fungi.



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