RESIPATH
Response of European Forests and Society to Invasive Pathogens
Contract - BR/132/A1/RESIPATH-BE

Summary

In the RESIPATH project we studied how European forest communities have been affected by and responded to invasive fungal pathogens. This report focuses on the contributions of the Belgian partners, who were involved in two work packages.

The first work package related to *Phytophthora* hybrids. We studied the prevalence of *Phytophthora* hybrids in different environments (forests, rivers, nurseries) and across European climatic regions because we hypothesized that their presence and invasion in such environments is increasing, in part due to the growing international trade in nursery plants. Those plants may be latently infected, thus creating a pathway for the introduction and mating between *Phytophthora* species that were previously geographically isolated. Hybrids can have increased pathogenicity and host range due to the combination of the parental genetic traits, which may result in invasive behavior. The main objective was to develop sensitive methods to detect *Phytophthora* hybrids and to apply these methods to a collection of isolates from the different environments and geographical origins. We optimized, validated and used two methods, GBS (Genotyping By Sequencing) and flow cytometry, on a collection of 836 *Phytophthora* isolates. The GBS technique gave unprecedented sensitivity and was cost effective. We identified a considerable number of isolates as hybrids, in several cases for the first time. We could often also determine which isolates of the parental species in our database were related to the observed hybrids. In other cases it was clear that one of the parental species has not yet been described. The second technique, flow cytometry, helped in the identification and characterization of allopolyploid hybrids. Although the analyses are still ongoing, it would appear that hybrids are especially present in specific *Phytophthora* clades, and are mostly found in the environments in which the isolates of those clades are endemic. So far, we could not find support for our hypothesis regarding a higher prevalence of hybrids in nurseries.

The second work package in which the Belgian partners were involved focused on the early detection of aerial spores of potentially invasive forest pathogens. Using qPCR and optimized DNA extraction protocols, we first compared three spore trapping systems (filter paper, Burkard volumetric sampler and a rotating-arm spore trap (rotorod-style)) for their efficiency in the detection of three forest pathogens displaying different spore sizes and belonging to different taxa. We then combined the best trap (Rotorod samplers) for sampling in the forest environment with an NGS amplicon sequencing protocol and a new bioinformatics pipeline allowing identification to species level. The system was first validated on mock communities of fungi. We applied this protocol to DNA samples extracted from Rotorod samplers put in different forests stands and compared the results with those obtained with qPCR for the three forest pathogens used as fungal markers. The NGS technique was less sensitive than real-time PCR but it allowed the detection of numerous other fungi, among which tree pathogens.

Our contribution in both work packages improved the ability to detect potentially invasive fungal pathogens, which should allow us to respond to them faster and more appropriately and help mitigate their impact.

Key words: Flow cytometry, Genotyping by sequencing, High throughput sequencing, Invasive fungal pathogens, *Phytophthora* hybrids, spore trapping.