Dieback of European ash is currently one of the greatest ecological emergencies on our continent. In certain forests, more than 80% of ash trees will die because of this disease, which is caused by a pathogenic fungus *Hymenoscyphus fraxineus*. Since the first reports of ash dieback in Polish forests in 1992, progress has been made in understanding the biological basis of the disease, however, the molecular processes underlying the dieback are still not fully established. Specifically, we do not understand how the plant cell wall – a polysaccharide barrier protecting each ash cell from infection - is degraded by the pathogen to allow its growth within tree tissues. Moreover, we do not know if and how ash trees detect the fungal pathogen and if this detection allows for triggering of immune response in ash cells. By answering these questions, we will be able to identify new routes to breeding ash trees resistant to dieback. This will enable maintenance of this iconic species in European woodlands.

To investigate the molecular basis of ash dieback, the project, performed in collaboration with the University of Cambridge and the University of Warwick in the United Kingdom, will study the changes in cell wall polysaccharides associated with infection of ash cells with the *Hymenoscyphus fraxineus* pathogen. Firstly, we will analyse how the cell wall changes in response to the fungal pathogen in trees grown in different forests in Poland. Leaf and wood samples will be collected from healthy, diseased and disease resistant ash trees and biochemical and genetic analyses will be performed to identify which sugars are changed during pathogen attack and which cell-wall and immunity related genes are activated by the tree during this process. Secondly, the project will use ash cell cultures to recreate the infection process by *Hymenoscyphus fraxineus* in a Petri dish. Through collaboration with the University of Cambridge and The University of Warwick we will monitor changes in the cell walls of cultured ash cells using state of the art approaches such as confocal microscopy, mass spectrometry and solid state Nuclear Magnetic Resonance. Full use of these powerful research techniques is only possible when ash cell cultures are analysed. Moreover, this year we reported first successful genetic engineering of cultured European ash cells. We propose to use this breakthrough discovery to modify the polysaccharides present on the surface of ash cells to render them resistant to infection by the *Hymenoscyphus fraxineus* pathogen. This will enable us to establish if changes in cell wall polysaccharide structure are a feasible route for breeding ash trees protected from dieback. Finally, we will also study the *Hymenoscyphus fraxineus* pathogen itself. We will use biochemical and genetic analyses to establish if polysaccharide fragments of fungal cells trigger immune response in ash and to analyse what molecular machines, known as glycosyl hydrolase enzymes, allow the fungus to infect tree cells.

European ash is a tree species with broad geographical distribution and high-quality timber. These properties make ash both ecologically and economically important. Therefore, the study of ash dieback is of significant public interest. The disease is having a devastating impact on European woodland ecosystems, with high mortality rates in most forests. Interestingly, in most studied populations some individuals are largely resistant to the disease. The basis for this resistance remains poorly understood and the need to fill this knowledge gap is one of the key scientific reasons for undertaking this research project. We hypothesise that the resistance of some individuals may originate, at least partially, from the molecular structure of the polysaccharide cell walls that surround ash cells. By testing this hypothesis, we may be able to pinpoint cell wall features that may prevent disease development in ash. The need for identifying these resistance-determining molecular features, which may act as future tree breeding targets, is another reason for undertaking this research study.

The unique blend of expertise in our interdisciplinary research team and the ability to genetically modify cultured ash cells are key advantages of this project. Through holistic analysis of ash cell wall and genetic modification of cultured ash cells, we will develop an unprecedented understanding of the importance of polysaccharides in ash dieback. Capacity for genetic modification makes our disease model applicable in a range of studies looking at dieback and will therefore likely accelerate research of multiple scientific groups worldwide. By investigating, together with scientists from the University of Warwick and the University of Cambridge, if and how modification of cell wall structure participates in tree response to pathogens, we hope to advance research in plant pathology since our discoveries may also be applicable to other tree diseases, not only to ash dieback. As such, our project may enable identification of breeding targets for development of pathogen resistant tree varieties which may safeguard woodland ecosystems for future generations.