Implementation of a new rapid sensitivity test to *Eutypa lata*, for the selection of more tolerant grape varieties and clones.

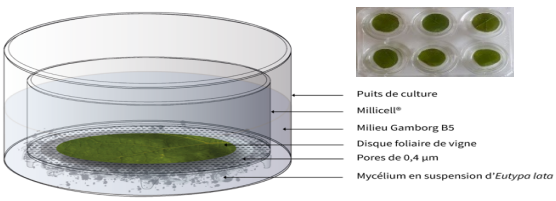
"Eutypa test" project.

The objectives of the "Eutypa test" project are the transfer to the laboratory of BNIC Station Viticole of rapid diagnostic methods, and the development and application of these tools to measure the sensitivity to *Eutypa lata* of new grape varieties and clones.

This transfer of technology required the acquisition and handling of equipment to ensure molecular biology analysis. These analytical tests have been adapted and optimized to facilitate their application to the activity of a field laboratory. Staff have been trained in these new technologies.

These new tools are:
- an in vitro infection test on leaf discs, called "Eutypa test", based on the expression of genes involved in the vine's response (marker genes),
- a specific and quantitative assay by qPCR of *Eutypa lata* in wood, correlating the sensitivity with the development of the pathogen,
- an easy-to-use statistical tool which relates the genotype of the grape varieties studied with databases of symptoms observation in the field (IFV).

The first of these tools is based on the study of sensitivity marker gene responses following an artificial in vitro infection of leaf discs taken from plants grown in pots in a greenhouse. This protocol uses a culture insert, called Millicell® (Millipore) which physically separates the leaf disc from the mycelium while maintaining a "molecular dialogue" allowed by a porous basement membrane. The molecules secreted by the fungus will therefore migrate through this membrane to the leaf, thus mimicking the circulation of toxins from the fungus to the leaves, through the sap of the vine. This accelerated infection makes it possible to study transcriptional responses by RT-qPCR after 3 days of co-culture (Cardot et al., 2019). The first promising results require some adjustments, in particular with regard to controlling the conditions for growing plants in pots in a greenhouse.



The second consists in measuring in the wood, 6 months and 1 year after an artificial infection of cuttings, the number of copies of certain genes, specific to *Eutypa lata*. The first interpretations of these results confirm and validate the protocol implemented. Fungal DNA is detected and quantifiable. In addition, this quantity can be related to the sensitivity of the grape variety or clone studied.

The IFV for its part has developed a statistical test intended to establish a correlation between the sensitivity, estimated by the observations of visual symptoms in the field, and the genotype of the individuals. It is implemented in relation with the TOLEDE project. For this, an executable program was developed with R statistical software. The first observations of symptoms were made on the plot of the progeny of an INRA-BNIC crossing. However, as the vines are still young (2015), they do not yet show enough symptoms of Eutypa dieback or other wood diseases. The data processing system is now ready for a larger data set to be used. .

The "Eutypa test" project therefore ended with the transfer of optimized technologies to BNIC Station Viticole laboratory, which is now operational. Progress has been made in understanding the sensitivity of the grape varieties and additional analyzes are needed to assess the sensitivity of the new BNIC grape varieties. The two methods developed are described in fact sheets which will be made available to interested laboratories.

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