In-field LAMP quantification of *Plasmopara viticola* airborne inoculum to improve the forecast of epidemic risk

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1 Introduction

Wine making is a very pesticide-consuming activity, especially in France (Urruty *et al.,* 2016). The European directive 2009/128 laid the foundations for a regulatory framework inviting State Members to set up plans to reduce the use of pesticides, i.e. Ecophyto II+ for France. Grapevine downy mildew, caused by the oomycete *Plasmopara viticola*, is one of the most devastating cryptogamic disease of grapevine worldwide. Protection against this pathogen is largely based on fungicide applications that accounted for 80% of total pesticides used on grapevines in France in 2016 (Simonovici, 2019). Reducing the number of applications and adapting the doses to the epidemic context are two levers for developing low-input protection strategies. Elaborating such management approach requires a better understanding and prediction of disease epidemics.
An usual way of progress is the predictive modelling of epidemic risks based on different biotic and abiotic variables, leading to the design of decision support systems, allowing adapted interventions to the pest pressure. The influence of abiotic factors on life cycle of *P. viticola,* including host infection (Williams *et al.,* 2007; Mouafo-Tchinda *et al.,* 2020) and epidemic spread (Rossi & Caffi, 2012; Brischetto *et al.,* 2020; Cortinas Rodriguez *et al.,* 2020) are well documented. The conditions for germination of primary inoculum (Rouzet & Jacquin, 2003; Rossi & Caffi, 2007; Rossi *et al.,* 2008) and asexual sporangia formation (Rumbolz *et al.,* 2001; Caffi *et al.,* 2013) are also well known. On another hand, the appearance of the first symptoms (Delière *et al.,* 2015), as well as the evolution of disease intensity are also used, most often in addition to weather data (Kennelly *et al.,* 2007), to design models systems (Tran Manh Sung *et al.,* 1990; Rossi *et al.*, 2008, 2009; Vercesi *et al.,* 2010; Brischetto *et al.,* 2021) and decision support (Caffi *et al.,* 2010).

Another way to measure the biological activity of a pathogen is the monitoring of its airborne propagules (Van der Heyden *et al.,* 2021). Carisse *et al.* (2005 & 2008) have shown the relevance of integrating such indicators in risk prediction and treatment modulation to control late blight onion caused by *Botrytis squamosa*. Airborne spore monitoring is already used as a qualitative indicator of the optimal positioning of the first treatment in the case of early detection of the pathogen (Thiessen *et al.,* 2016). Continuous monitoring of the pathogen spore concentration in the atmosphere can also constitute a parameter for modulating the treatment schedule or dose of active substances applied during ongoing season. (Thiessen *et al.,* 2017). Formally carried out by optical microscopy, the detection and quantification of micro-organism propagules is very time-consuming and therefore not well suited to the time scale of a farmer's decision making. Another way to analyse aerobiological samples is to target nucleic acid sequences (DNA or RNA), specific of the organism of interest (PCR based method). This molecular approach based on quantitative PCR, presents the advantage of being very sensitive, specific, and quantitative, in an overall shorter period.

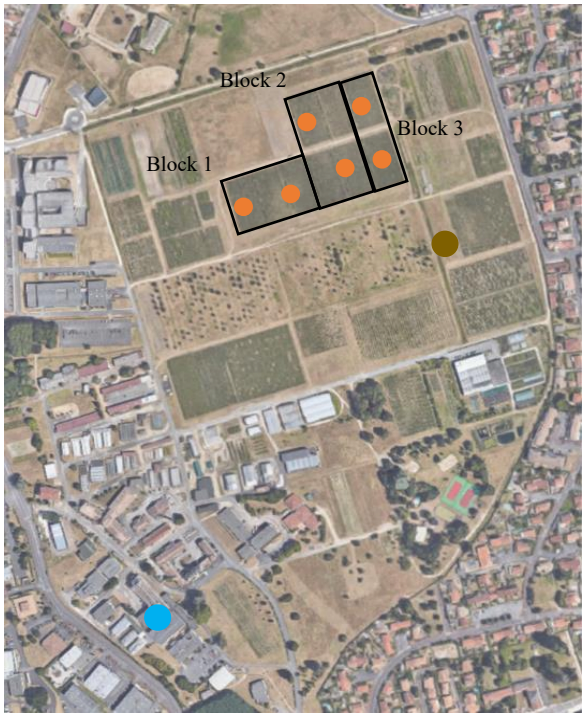

Figure 1: Study set-up on the experimental unit of INRAE Grande Ferrade experimental station (Villenave d'Ornon - France). Blue dot: building trap. Brown dot: litter trap. Orange dot: trap located within the canopy of the different plots.

2 Spore monitoring systems

Largely inspired by existing methods, we are developing a multispecies airborne spore monitoring system, initially directed against *P. viticola*, using vortex air samplers coupled with loop-mediated isothermal amplification (LAMP; Notomi *et al.,* 2000). Several types of traps, active and passive, have been developed for the analysis of airborne propagules such as spores and pollens. The choice of a particular method of trapping essentially depends on the purpose of the tests, work hypothesis and environmental conditions more than on the performance of the trapping

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system itself (Hirst, 1995; McCartney *et al.,* 1997). A rotorod sampler, the Sporestick (OptiSense Ltd.) was selected, which is an active impaction trap, sometimes also called vortex air sampler. This type of trap is easy to use on a routine basis for an attractive price. The collection matrix used (two matches coated with petroleum jelly) is suitable for both molecular biology analyses and light microscopy counts. Moreover, the collection of spores is little affected by wind speed or particle size, when their diameter is greater than 10 µm (Atkinson *et al.,* 2018; Torfs *et al.,* 2019). Recently, the Sporestick has shown very good performances in terms of sensitivity compared to other active and passive traps, for the continuous monitoring of sugar beet powdery mildew, caused by the ascomycete *Erysiphe betae* (Pizolotto *et al.,* 2021).

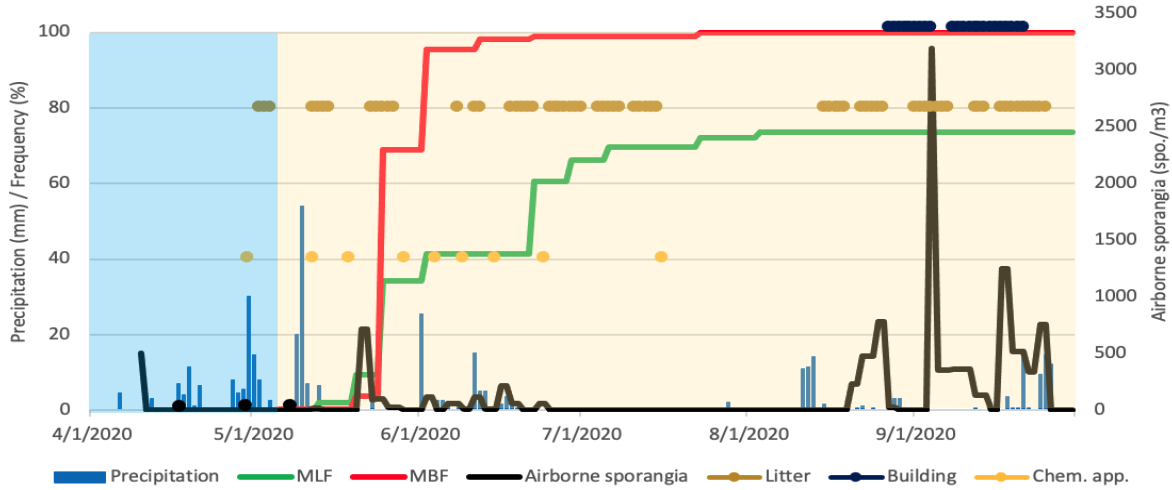
Detection or quantification of *P. viticola* by quantitative PCR is of common use (Valsesia *et al.,* 2005; Carisse *et al.,* 2020). LAMP is another molecular technique that is based on a more complex set of primers than conventional PCR-based methods. It has the advantage of starting the detection with small number of DNA and provides accessible, cost-effective, and easy-to-perform method. LAMP assays have been successfully used on airborne environmental samples for the detection of grapevine powdery mildew caused by *Erysiphe necator* (Thiessen *et al.,* 2018), sugar beet rust caused by *Uromyces betae* (Kaczmarek *et al.,* 2019), cereals eyespot caused by *Oculimacula acuformis* and *O. yallundae* (King *et al.,* 2021), potato and tomato late blight caused by *Phytophthora infestans* (Arocha Rosete *et al.,* 2021). A method for detecting sporangia of *P. viticola* by LAMP protocol on the ITS-2 sequence has also been developed by Kong *et al.* (2016). Using these primers, we assessed a simplified extraction of nucleic acids from aerobiological capture samples carried out in the vineyards, followed by LAMP detection. By comparing our results with results obtained with the Digital Droplet PCR, we are able to validate the quantification nature of this simplified LAMP protocol (ddPCR ; Ristaino *et al.,* 2019).

The technique has been validated in our laboratory (Douillet *et al.,* submitted). It has now to be tested under real production conditions with uncontrolled natural pathogen pressure. This has been done on different experimental set-ups (Figure 1) in 2019, 2020 and 2021: environmental spore samples were collected three times a week on Monday-Wednesday-Friday, during the winegrowing seasons; weather and sanitary data have been recorded on these periods.

The first objective of our study was to focus on the early detection of primary inoculum. In order to optimise the probability of capture of the germinated oospores (overwintering forms of *P. viticola*), an artificial litter of downy mildew-contaminated debris was made, over which continuous trapping was maintained from March to October. Each year, *P. viticola* DNA was detected above the litter before leaf symptoms appeared in the vineyards, sometimes very early in the season (before stage 4 Eichhorn and Lorenz scale). Several airborne spore peaks were detected independently of precipitation events, prior to symptom onset, indicating that macrosporangia germinating from oospore can be disseminated by the air as well as splashing due to rain, which is the most commonly way of dissemination usually described.

According to the specificities of *P. viticola* life cycle, a hypothetical circadian rhythm and a possible vertical gradient on a row scale of sporangia emission were tested in order to define the best in field trapping protocol. The daily trapping period was divided into six 4 hours periods. Though the sporangia emissions do not last longer than one time slot period of 4 hours per day, it has not been possible to define which single period of 4 hours could be enough to characterize the whole trapping of a full day. In order to

Figure 2: Example of time series generated in 2020 (airborne sporangia from plot in block 1). Blue bar: precipitation (mm); Green line: downy mildew leaf incidence (%); Red line: dony mildew bunch incidence (%); Black line: airborne sporangia concentration (sporangia/m^3); Black dot: detection of airborne sporangia (masked by noise); Brown dot: detection above the litter device; Blue dot: detection on the building device; Yellow dot: chemical applications. The light blue rectangle symbolizes the pre-symptomatic period. The yellow rectangle symbolizes the post-symptomatic period.



ensure that trapping times are exhaustive and to avoid missing any emission events, continuous trapping day and night is still recommended. In the same way, the location of the spore trap at the row height within the canopy was tested: the most relevant capture position is just above the canopy, as already proposed by Carisse *et al.* (2017) for monitoring the airborne spore of *Erysiphe necator*.

In parallel, continuous captures on 6 plots divided into 3 blocks of the Grande Ferrade experimental station (INRAE, Villenave d'Ornon) were carried out during the vineyard season, from April to October. These 22 are of cv. Merlot plots, are part of a 2 hectares patch. The main purpose of this network of 6 spore traps is to evaluate the heterogeneity of the sampling at the plot scale. A plot heterogeneity is indeed observed, with quantities of sporangia captured really correlated to the very close sanitary state of the plot during the post symptomatic phase. In the pre-symptomatic phase, the low frequency of capture events makes it difficult to statistically analyse the results, even if globally, on each plot and each year, positive captures of sporangia are detected during this period. Figure 2 illustrates this emission pattern for the year 2020 in one plot of block 1. During the pre-symptomatic period (blue), emission peaks are detected in the plot on 11 April (black line and dots) and above the litter on 03rd May (brown dot) when the first symptoms are observed on the 06th of May. Thereafter, a rather explosive development of downy mildew on bunches (Mildew Bunch Frequency; MBF red line) and leaves (Mildew Leaf Frequency; MLF green line) are observed. They clearly seem to be correlated to the emissions of spores collected until early July. At the end of the season, one month after the last treatment applied on the 16th of July at bunch closure stage (orange dots), we can notice, almost one month later, a strong increase in airborne sporangia concentration. This downy mildew mosaic propagation on the leaves is very usual on the Bordeaux vineyard each end of seasons, because of high moisture and presence of dew on the leaves quite every night.
A Sporestick has been placed nearby on a building roof, 15 meters above ground level in order to assess whether if we can observe an inter plots spore transfers out of the vineyard at a higher altitude. Our results suggest that a long-distance transport might mix *P. viticola* populations. We can also observe that the detection on this high device (blue dot) is synchronised with the massive level of capture within the field. In the same way, we can observe on the two other years of our experiment (2019 and 2021) that the atmosphere becomes heavily loaded with airborne sporangia, each end of winegrowing seasons. Turbulences and convection air movements carry the sporangia to higher altitudes where they can be captured by the device, 15 meters above the ground.

3 Conclusions

These results allowed us to specify an optimal capture protocol that has been used to set up a first network in Bordeaux vineyard, opening the way to regional airborne spore based epidemio-survey.

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