Highly sensitive spore detection to follow real-time epidemiology of downy and powdery mildew

Leoni S[1,2*], Basso T.[2], Tran M.[2], Schnée S.[1], Fabre A.-L.[1], Kasparian J.[2], Wolf J.-P.[2], Dubuis P.-H.[1]

[1] Mycology group, Research Department Plant Protection, Agroscope Changins, Nyon, Switzerland
[2] Department of Applied Physics, University of Geneva, Geneva, Switzerland

1 Introduction

The oomycete *Plasmopara viticola* (PV) and the ascomycete *Erysiphe necator* (EN) are the major threat in grapevine diseases, causing respectively downy and powdery mildew. As polycyclic pathogens, both have a fast asexual cycle leading to a high production and release of spores in the environment (Viret and Gindro, 2014). Wind plays the primary role, but little is known about the distance or the duration of the aerial dispersion (Brischetto *et al.* 2021).
To counteract the attack of these pathogens, growers can rely on decision support systems tools predicting the probability of infections and allowing them to spray preventively at the right moment (Rossi *et al.,* 2008; Dubuis *et al.,* 2012; Legler *et al.,* 2014). Most of these forecasting models are based exclusively on environmental parameters, and as biological systems, some infections may go under the radar. Adding a biological parameter such as the spore concentration in the air maybe very useful to predict more precisely primary and secondary infections, and to better understand the seasonal epidemiology by following spatial variability of spores.
For this purpose, we are developing a spore detector device allowing us to count independently and in real time the number of PV and EN spores.

2 Preliminary assay in Dardagny

Before the construction of the spore detector, a first assay was conducted from June to October 2018 in a vineyard in Dardagny (Geneva, Switzerland)(Basso *et al.* 2020). Five stations were installed, distributed in ca. 50 ha with an average distance of 400 m between them. Each station was equipped with an optical particle counter able to detect particles based on their size as they pass through a laser beam. The data were compared to reference measures made by adhesive tapes on passive spore trap for 7 days and observed by multimodal and multiphotonic microscopy (Bellow *et al.* 2012; Kilin *et al.* 2017), confirming the correspondence between particles and PV spores. We observed during October 2018 that the concentration of particles corresponding to PV spore size (16-20 µm) were fluctuant from one station to another (Fig. 1). Moreover, no temporal correlation of high episodes of sporulation was observed, even between nearby stations. The sporulation of secondary infections seemed specific to the station and the dispersion did not seem to influence infections to other places. Temporal differences in sporulation could indicate that climatic factors of the microenvironment around the station could play a crucial role for the infection. This heterogeneity of spore concentrations within the same field highlights the importance of having multiple stations to finely measure sporulation.

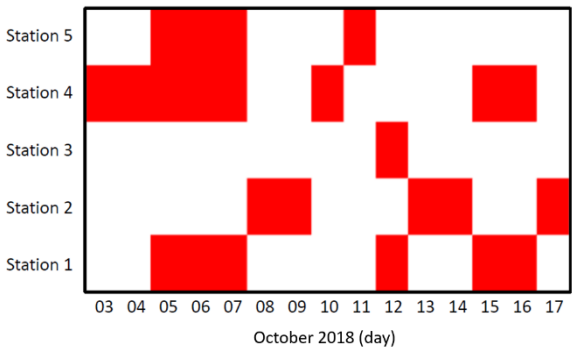

Figure 1: Days of high concentration of particles (16-20 µm spheroids), corresponding to the size of *Plasmopara viticola*, on 5 stations placed in Dardagny.

3 Spore detector and holography imaging

SMALA (SMart Agriculture using Lasers and Artificial intelligence) project is a collaboration between the Department of Applied Physics of the University of Geneva and Agroscope, the National Centre for agricultural research of the Swiss Confederation. The project aims to develop a low-cost and autonomous spore detector able to unequivocally detect PV and EN spores (Fig. 2). These solar powered and cost-effective devices use Rapberry Pi computers and cheap smartphones to take photos every 2 hours. Spore identification is based on holographic images produced when the laser beam hits the spores on a thin sapphire plate. Pictures are sent to analysis in real-time by artificial intelligence algorithms, trained to recognize and count PV and EN spores. We expect that digital holography will allow us to obtain with cost effective materials, very precise spore prints, unique to each species.

*Corresponding author: sara.leoni@unige.ch

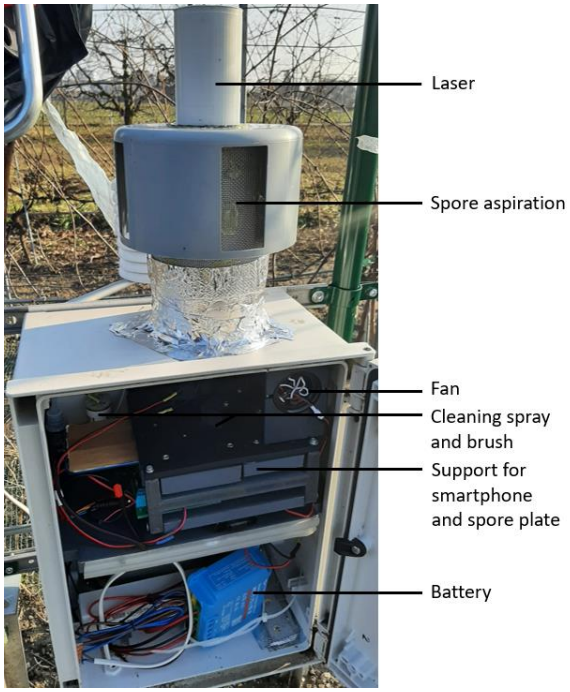

Figure 2 : Picture of the spore detector installed in field (Changins). The device measures approx. 40 x 25 x 60 cm (L x W x H).

4 Method validation by qPCR during season 2021

Spore detectors were deployed during 2021 in Changins (Nyon, Switzerland) in parallels of passive spore traps with tape, renewed once a week. Correlation of spore concentrations from the detector and the passive spore trap is made using multiplex qPCR on DNA extracted from tapes. A 4-plex qPCR was developed combining PV *aestivalis* (Carisse *et al.* 2021), EN, *Botrytis cinerea* and an exogenous internal positive control primers and probes originally designed by Carisse *et al*. (2014). The comparison between both imaging and molecular techniques will allow us to confirm the efficiency of this new spore detector.

5 Conclusions

We are at the beginning of this project and first results will be presented during the GDPM 2022, although preliminary assay showed that this innovative device is promising and could be extended to other pathogens. The quantity of spore inoculum in the air would be a new input in the already existing forecasting model VitiMeteo-Plasmopara (Dubuis *et al*, 2012) to increase the accuracy on infection prediction. The aim is to spray only when the risk of infection is significant, lowering the use of pesticide. The information on the heterogeneity of the spore inoculum inside the vineyard in real time will allow us as well to follow the epidemiology of each disease and treat in an even more targeted manner. Thus, this low-cost device will allow us to reduce the number of treatments to the strict necessary.

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