# Oospore germination dynamics and disease forecasting model: an integrated approach for downy mildew management

Maddalena[1,2*], G., Lecchi[1], B., Serina[3], F., Torcoli[3], S. and S.L. Toffolatti[1]

[1]Università degli Studi di Milano, Dipartimento di Scienze Agrarie e Ambientali, via Celoria 2, 20133, Milano, Italy
[2]Università degli Studi di Milano, Dipartimento di Bioscienze, via Celoria 26, 20133, Milano, Italy
[3]Ufficio Tecnico-Consorzio per la tutela del Franciacorta, via G. Verdi 53, 25030 Erbusco (BS), Italy

## 1 Introduction

Downy mildew, caused by *Plasmopara viticola* (Berk. *et* Curt.) Berl. e De Toni, is one of the most economically impacting disease of grapevine (Bois *et al.,* 2017). In absence of an adequate control, the disease leads to severe yield losses (Gessler *et al*., 2011; Toffolatti *et al.,*2018). Currently, fungicide application to control grapevine downy mildew starts early in spring and is frequently repeated during the vegetative season, resulting in a large number of treatments with plant protection products (PPP), with implications on health, environment and production costs.
In the new framework of the Farm to Fork Strategy of the European Green Deal, the European Commission will take additional action to reduce the overall use of chemical pesticides by 50% by 2030. Currently, with 40% share (Eurostat, 2021), fungicides are the most sold group in the EU. Therefore, to achieve the goal of the Farm to Fork Strategy, a drastic decrease in the number of fungicide applications should be accomplished in a relatively short period of time. A key point in improving the effectiveness of grapevine downy mildew control strategies consists in determining the right timing for PPP schedules, which is still difficult for primary infections, to avoid the application of unnecessary treatments. As a consequence, more efforts and researches are needed with the aim of developing novel solutions to achieve a sustainable disease management.
*P. viticola* is a biotrophic and obligate parasite and a polycyclic pathogen, causing both primary and secondary infection cycles (Figure 1), The pathogen survives in absence of the host by differentiating resting structures, the oospores. These survival structures, originated by sexual reproduction, produce the inoculum for primary infections. The oospores are formed into the host tissues and overwinter on the surface litter. Generally, the oospores germinate in spring, differentiating a macrosporangium at the apex of germ tube, where zoospores, the infection spores, are formed (Vercesi *et al*., 1999). Therefore, primary infections occur in consequence of the oospore germination when the zoospores reach susceptible grapevine tissues and infect the host through stomata (Figure 1). The oospores represent a source of inoculum during the entire host growing season and, frequently, overlap secondary inoculum (Gobbin *et al*., 2005). Secondary infection cycles are caused by the asexual inoculum, consisting of zoospores differentiated by sporangia.

The main ecological factors influencing the oospore germination process are temperature (Ronzon-Tran Manh Sung and Clerjeau; Burruano *et al.,* 1990) water (Burruano *et al.,* 1987; Rossi and Caffi, 2007), soil humidity (Burruano *et al.,* 1992), and location (Galbiati and Longhin, 1984; Burruano *et al.,* 1989) alone or in combination (Ronzon-Tran Manh Sung and Clerjeau, 1987; Rossi *et al.,* 2008; Vercesi *et al.,* 2010). Relating the influence of environmental conditions to the germination dynamics could provide indication on inoculum availability for primary infections and the need for a fungicide treatment. A recent study demonstrated that the number of days required by the oospores to germinate *(t)* decreases when grapevine reaches susceptibility to *P. viticola* (Maddalena *et al*., 2021).

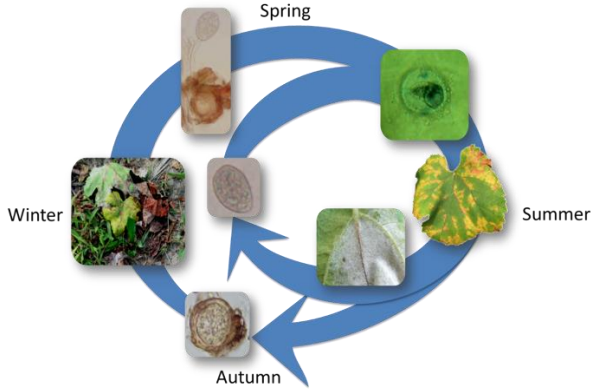


Figure 1: Life cycle of *Plasmopara viticola*, the causal agent of downy mildew of grapevine.

Many epidemiological models have been developed to identify which conditions favour the disease development (Rossi *et al*., 2008; Caffi *et al*., 2011; Legler *et al.,* 2011; González-Domínguez *et al.,* 2011; Orlandini *et al*., 1993; Rodríguez-Rajo *et al.,* 2010; Hill *et al.,* 2019). EPI (Etat Potentiel d'infection), the heuristic model designed for the assessment of *P. viticola* infection, was formulated by Strizyk in 1981 and thanks to numerous experiments carried out subsequently on grapevine downy mildew in France and in Northern Italy (Fremiot *et al*., 2008; Parisi *et al*., 2012), the EPI algorithm has undergone a series of modifications, also enriching with the results of the research carried out on the oospore germination (Vercesi *et al*., 1999). The model, developed by "Sesma", is currently included in the Epicure system adopted by the Institut Français de la Vigne et du Vin

*Corresponding author: giuliana.maddalena@unimi.it

(IFV) (https://oadex-viti.vignevin-epicure.com). The promising results obtained in the previous years led the authors to evaluate the effectiveness of EPI model extending its use in other viticultural area.
This study aimed at combining biological data on the oospore germination with the use of the EPI model to estimate the risk of infection in Franciacorta, an important Italian viticultural area located in province of Brescia.
The analysis on oospore germination data were carried out on three populations of *P. viticola* oospores overwintered in natural field conditions. The disease forecasting model was used in ten vineyards. The results obtained with the oospores and the model were compared with the real epidemics in field, by estimating disease incidence and severity (Figure 2).

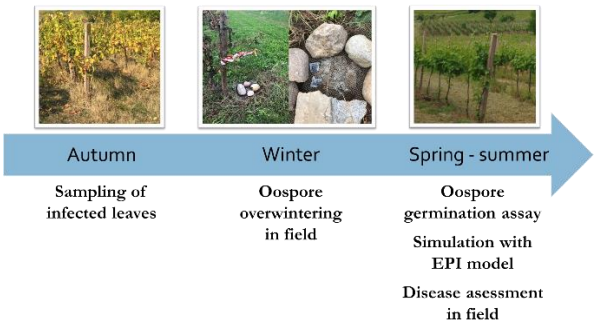


Figure 2: Experimental procedure pattern

## 2 Materials and methods

### Vineyards

Experimental activities were carried out in 2021 in ten vineyards located in Franciacorta, in province of Brescia (Northern Italy, Lombardy region). Meteorological data (hourly temperatures, rain and relative humidity) were collected throughout *in situ* weather stations (Figure 3, Table 1). In each vineyard, a plot consisting in 3-4 rows (75 plants on average) was not treated against *P. viticola,* to assess the epidemic development weekly.

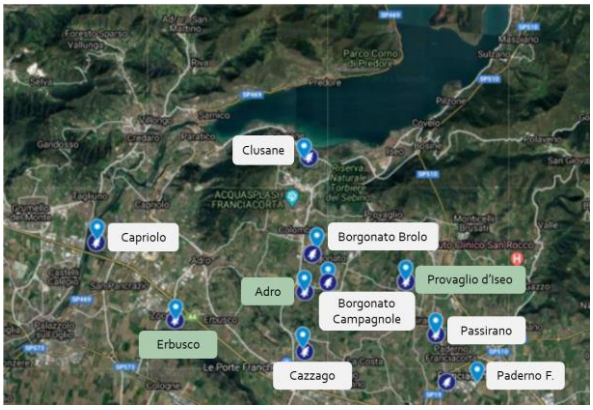


Figure 3: Map of the province of Brescia representing locations of weather stations and vineyards

| Comune | Locality | Cultivar | Training system |
|---|---|---|---|
| **Adro** | Formica | Bacca rossa | Guyot |
| **Corte Franca** | Borgonato Brolo | Chardonnay | Guyot |
| **Corte Franca** | Borgonato Campagnole | Chardonnay | Guyot |
| **Capriolo** | Cascina Bosco Basso | Chardonnay | Guyot |
| **Cazzago San Martino** | Calino | Chardonnay | Guyot |
| **Clusane** | - | Chardonnay | Guyot |
| **Erbusco** | Sottomonte | Chardonnay | Guyot |
| **Paderno Franciacorta** | Colombaia | Chardonnay | Guyot |
| **Passirano** | Prada | Merlot | Spurred cordon |
| **Provaglio d'Iseo** | Roccolo | Chardonnay | Guyot |

Table 1: List of the vineyards with relative agronomic characteristics

### Oospore germination dynamics

The oospore germinations dynamics was evaluated for three vineyards located in Adro, Provaglio d'Iseo and Erbusco.
Grapevine leaves, showing downy mildew mosaic symptoms, were collected at the middle of October 2020. Twenty nylon bags containing leaf fragments rich in oospores were prepared (Maddalena *et al.,* 2021). Germination assays were carried out once or twice a week starting from grapevine sprouting (second half of March 2021) until bunch closure (end of June 2021) as described by (Maddalena *et al*., 2021). For each nylon bag, three agar plates (technical replicates) were inoculated with four 10 µl droplets of 100 oospores and incubated at the optimal temperature of 20°C. Macrosporangium formation was checked daily at the stereomicroscope (Leica Wild M10) from 1 to 16 days after incubation (dai). These data were used to calculate, for each germination assay, the minimum number of days required by the oospores to germinate (*t*) (Maddalena *et al*., 2021).

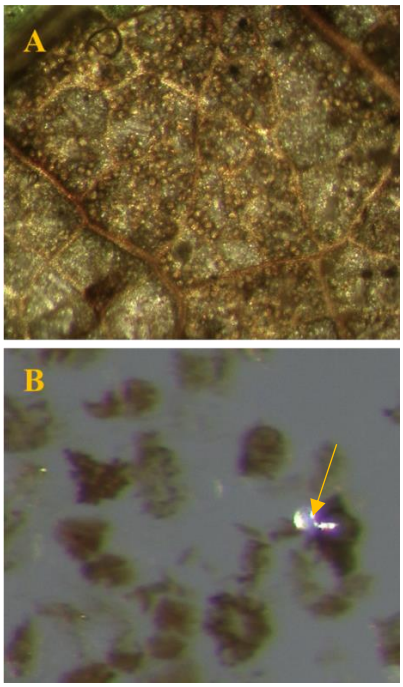


Figure 4: (A) Oospores in leaf tissue visualized through stereomicroscope; (B) Germinated oospore visualized through stereomicroscope

**Disease epidemics simulation with EPI disease forecasting model**

Weather data were recorded from September 2020 by meteorological stations located in each vineyard. Weather forecasts over next five days were used by the model to predict the infection risk. Minimum and maximum values of temperature (°C), relative humidity and hourly amount rainfall (mm), were imported in the EPI model. Based on weather data, the model calculates FTA index (Theorical Frequency of Attack), that provides indications on infection risk.

The downy mildew epidemic development was assessed weekly in the untreated plots, consisting of 75 plants on average, starting from the first symptoms appearance. Leaves and bunches were carefully observed for downy mildew symptoms and disease incidence (I%D) was calculated as a percentage of affected leaves or bunches. When infection resulted heavy in the untreated plots, the disease severity was estimated through the calculation of percentage index of infection (I%I) (Townsend and Heuberger, 1947; Toffolatti *et al*., 2018). The average I%I of leaves and bunches have been obtained from the individual values calculated for the three subplots.

Moreover, the length of incubation period was calculated (Goidanich *et al*., 1957), to evaluate ascertain the most probable date of disease infection occurrence.

Weekly bulletins reporting information about infection risk, the oospores germination assays and the real epidemic development observed in field, were provided to the farmers.

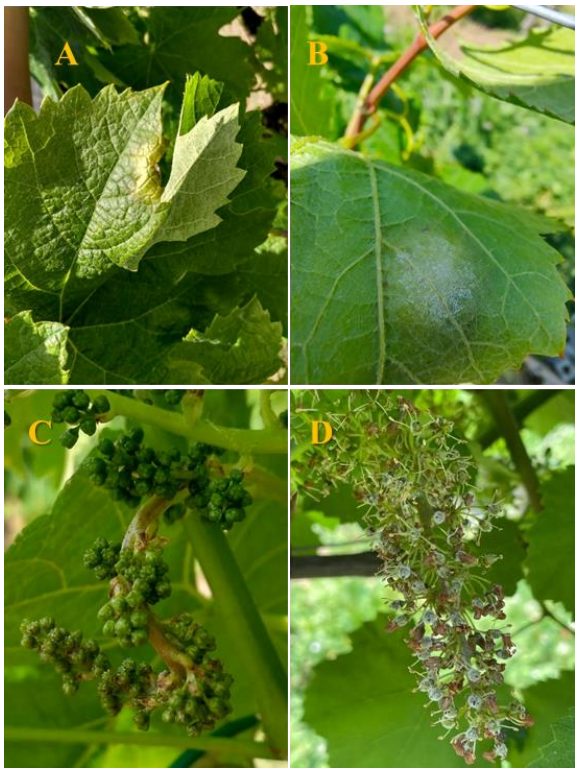


Figure 5: (A) Symptom on leaf; (B) sporulation on leaf; (C) symptom on bunch; (D) sporulation on bunch

**3 Results and discussion**

Overall, the EPI model predicted a medium-high infection risk since the end of April to the first ten days of June and, in some cases, at the beginning of July. The first symptoms in field were observed between 6$^{th}$ and 21$^{st}$ of May as a result of infection that occurred between 28$^{th}$ of April and 11$^{th}$ of May, as predicted by the model. Interestingly, in correspondence with the infection risk indicated by the EPI model, the oospores showed a reduction in *t* (with minimum values ranging from two to five days). This confirms that *t* significantly decreases when the plant reaches susceptibility to *P. viticola* (Maddalena *et al*., 2021).

It must be point out that, since the oospores are the only overwintering structures of the pathogen and the source of the vital inoculum for primary infection, obtaining real biological data on oospore germination is particularly important also from an epidemiological point of view since it provides information on the availability of inoculum for primary infections. Indeed, the germination of the oospores monitored in laboratory, was observed until 21$^{st}$ of June, indicating the possibility of primary and secondary infections overlaps during the season. The results obtained by field evaluation carried out weekly on untreated plots showed a slow but progressive increase of the disease until the middle of June. Therefore, the conditions in 2021 resulted favourable for disease development, as demonstrated by the medium-high average values of disease severity and incidence observed in the untreated plots, reported in Table 2.

| I%D | | I%I | |
|---|---|---|---|
| **Leaves** | **Bunches** | **Leaves** | **Bunches** |
| 66 | 68 | 18 | 31 |

Table 2: I%I and I%D average values for leaves and bunches at bunch closure.

The *a posteriori* evaluation of the model, highlighted a high accuracy of FTA index, calculated by the EPI model, in identifying periods when conditions were favourable for grapevine downy mildew development. Indeed, the FTA index correctly described the epidemic trend of grapevine downy mildew over the vegetative season that was investigated by field observations.

## 4 Conclusions

To conclude, the oospore germination assay, integrated with the forecasting model, allowed to identify the time window for the occurrence of infection during the whole vegetative season. Overall, the adoption of the EPI forecasting model combined with the analysis of oospore germination dynamics could contribute to definition of a rational treatment strategy, by identifying the right moment for the fungicide applications.
However, the high variability of grapevine downy mildew incidence across years, highlights the necessity of considering several seasons to validate the model. To this purpose, the present study will be repeated in the next vegetative season in the same vineyards.

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