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# Exploring the microbiota to identify antagonists of grapevine downy mildew

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We investigated the microbial communities of vineyard plots that are part of a grapevine disease monitoring network. We show that several species of fungi and bacteria are more abundant in the leaves and soil of vineyard plots historically less affected by downy mildew, a disease caused by the oomycete *Plasmopara viticola*. These microorganisms are promising candidates for the development of microbial consortia for biological control of downy mildew.

## Managing soil and plant microbiota as an alternative strategy for controlling downy mildew?

The oomycete *Plasmopara viticola* is an obligate biotrophic parasite and the causal agent of downy mildew, one of the major diseases affecting cultivated grapevines. This polycyclic pathogen infects leaves and grape clusters, causing substantial damage. Oospores, produced through sexual reproduction, form in the fall within infected leaves. After the leaves fall, the oospores persist in the soil and leaf litter, serving as the overwintering form of *P. viticola*. In the spring, the oospores germinate and develop a germination filament bearing a macrosporangium. This macrosporangium releases primary zoospores, which are dispersed by rain onto grapevines tissues and cause primary infections. After an incubation period, *P. viticola* sporulates on the surface of infected tissues and produces new zoospores, known as secondary zoospores. Several cycles of secondary infections can occur during the growing season and are responsible for the rapid spread of the disease. Disease management relies primarily on intensive fungicide applications. The VITAE research program (2020–2027)[1] aims to generate new knowledge to develop alternative approaches to vine protection, with the goal of reducing fungicide use. Manipulating the microbiota is one of the alternatives being studied.

Grapevines and vineyard soils host diverse microbial communities[2]. Several microorganisms, tested individually or in consortia[3], have been shown to interfere with the asexual reproduction of *P. viticola* through various antagonistic mechanisms. Furthermore, some studies[4] suggest that the soil microbiota may regulate the persistence and germination of overwintering oospores, opening up promising prospects for biological control.

The objective of our study[5] was to identify bacteria and fungi capable of interfering with the sexual and asexual reproductive cycles of *P. viticola*, in order to contribute to the development of biological control strategies against downy mildew based on microbiota management.

## Analysis of microbiota in plot pairs with historically contrasting downy mildew pressure

A field study[5] was conducted over two consecutive years in Nouvelle-Aquitaine (southwestern France) on pairs of vineyard plots included in a grapevine leaf disease monitoring network, coordinated by the French Institute of Vine and Wine (IFV). The plots within each pair exhibited contrasting incidence and severity of downy mildew symptoms over several years, even though they were planted with the same grape variety, were geographically close, and were subject to the same management practices (Figure 1A). Samples of young leaves and upper soil layer (0–5 cm) were collected from each plot (Figure 1B–C) in the springs 2022 and 2023. Upon arrival at the laboratory, we separated the epiphytic microorganisms, present on the leaf surface (phyllosphere), from the endophytes, present within the internal tissues (endosphere). Soil samples were homogenized and sieved prior to analysis (Figure 1D–E). The bacterial and fungal communities in the three sample types (phyllosphere, leaf endosphere, and surface soil) were then characterized using high-throughput sequencing (metabarcoding).

To identify microorganisms associated with low downy mildew pressure, we applied five complementary methods: four differential abundance analysis methods (ANCOM-BC2, MaAsLin2, LinDA, ZicoSeq) and a Random Forest (RF) machine learning algorithm. The RF algorithm was also used to evaluate the ability of microbiota data to predict disease pressure.

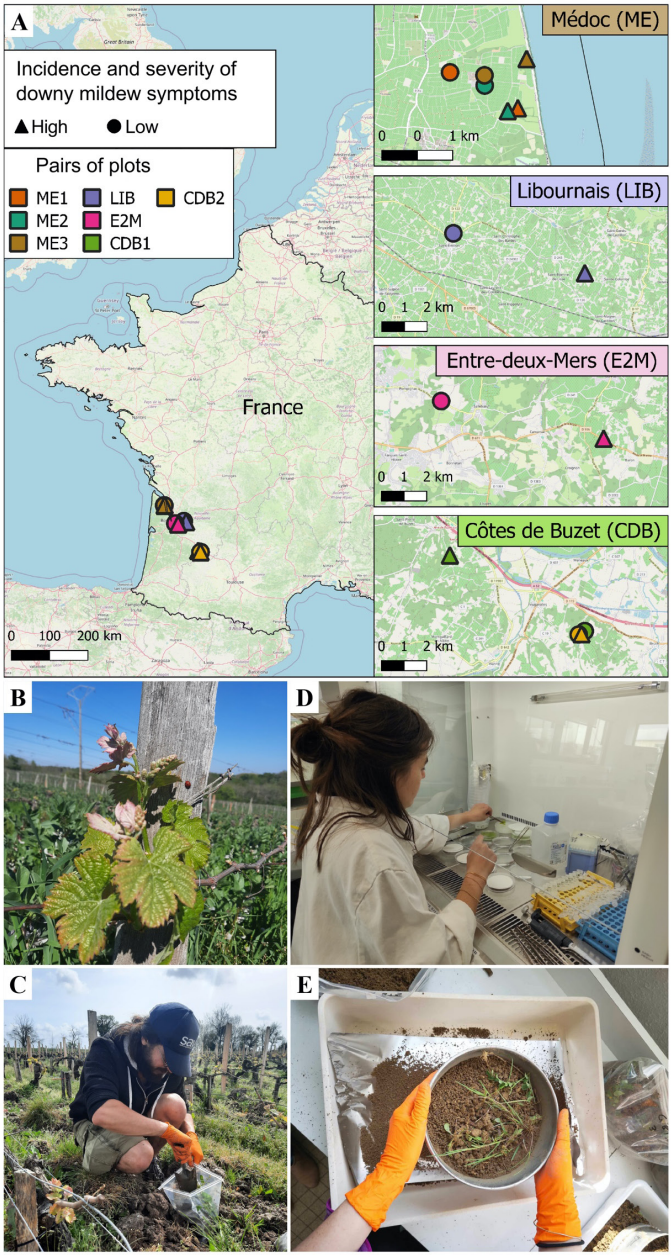



**FIGURE 1.** Experimental design[5]: (A) map of plot pairs selected based on epidemiological data on downy mildew; (B–C) leaf and soil sampling; (D–E) sample processing for microbiota analysis.


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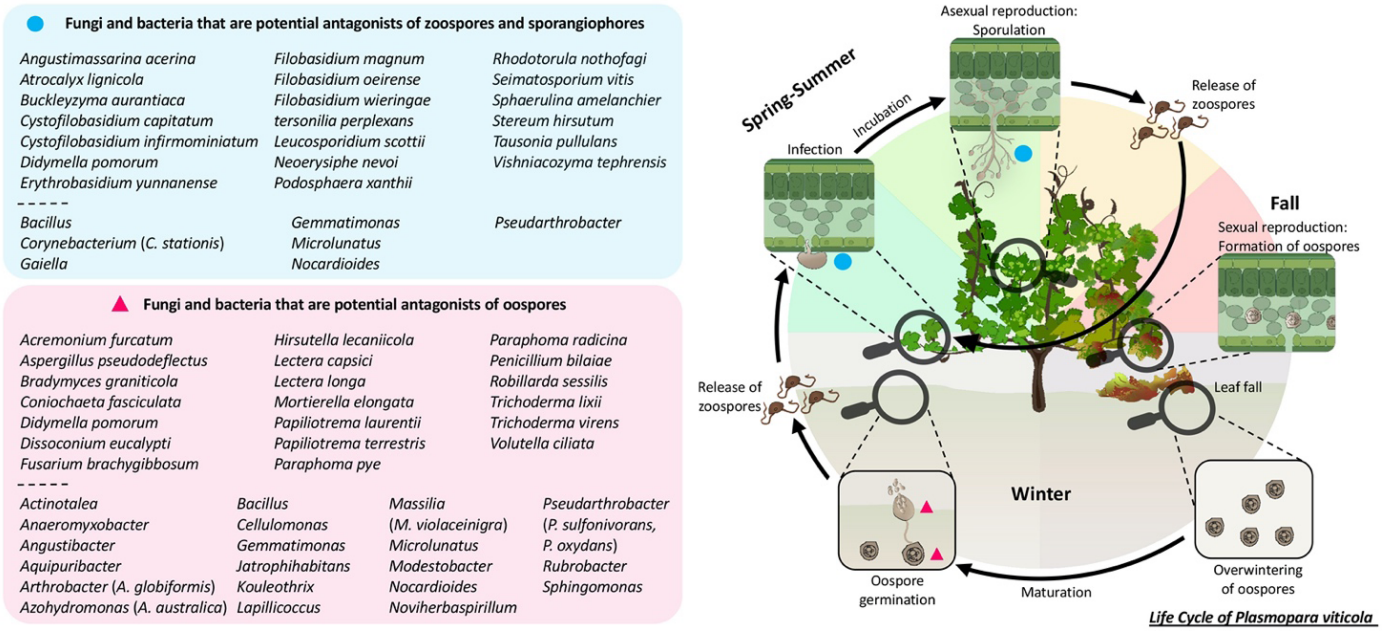



**FIGURE 2.** Fungi and bacteria from the soil and phyllosphere that may interfere with the sexual and asexual reproduction of the oomycete *P. viticola*, the causal agent of downy mildew in grapevines, at the start of the growing season[5].

## Several species of fungi and bacteria are more abundant in the soil and phyllosphere of plots with low downy mildew pressure

We identified microorganisms that were consistently more abundant in plots with low downy mildew pressure. They are found in the upper soil layer, where *P. viticola* oospores overwinter, and in the phyllosphere, where tissue infection by zoospores and sporulation following infection occur (Figure 2). In contrast, the leaf endosphere, where the mycelium of *P. viticola* grows, contained few microorganisms of interest. The surface soil and the phyllosphere therefore appear to be microbial reservoirs that should be prioritized for investigation in the development of biological control strategies for downy mildew. Figure 2 presents the most promising microorganisms for biological control.

## Basidiomycete yeasts are more abundant in the phyllosphere of plots with low downy mildew pressure

A large proportion of the fungi associated with the phyllosphere of plots with low downy mildew pressure are basidiomycete yeasts (Figure 2). Their role in biological control is well documented: several yeast species are already used to combat plant pathogens because they regulate their development through competition for nutrients and space, parasitism, or stimulation of the plant's defenses[6]. Some of these mechanisms could also be effective against grapevine leaf diseases, such as downy mildew. In the soil, the fungi characteristic of plots with low downy mildew pressure belong to genera known for biocontrol (*Trichoderma* and basidiomycete yeasts) or are new candidates (Figure 2).

## Several bacterial genera are more abundant in plots with low downy mildew pressure

As for bacteria, the taxonomic resolution of the molecular markers used did not allow for species identification in most cases. Figure 2 therefore shows the bacterial genera that were more abundant in plots with low downy mildew pressure. When bacteria could be identified to the species level, the species is indicated. Most of the bacterial genera associated with low downy mildew pressure were found in the soil.

## Downy mildew pressure in vineyards can be predicted from soil fungal communities

To identify the microbial communities most effective in predicting disease pressure, several subsets of data (fungi only, bacteria only, or both) were tested as predictors. The best predictions were obtained from soil fungi, suggesting a link between the composition of soil microbial communities and the incidence and severity of downy mildew symptoms on the above-ground parts of the vine.

## Toward the Development of Microbial Biocontrol of Downy Mildew

This study shows that, at the beginning of the growing season, several fungi and bacteria are more abundant in the leaves and soils of plots with low incidence and severity of downy mildew symptoms. Subsequent trials will aim to determine whether there is a causal link between these microorganisms and the disease, as well as the soil and climate conditions that naturally favor them. These microorganisms[7] have been isolated and are currently being evaluated in the laboratory and in the field, either alone or in combination, for their efficacy against downy mildew. These advances could lead to the development of new biocontrol strategies, through both the enhancement and conservation of beneficial microbial communities. Furthermore, the soil microbiota has proven to be a better indicator of disease pressure than the leaf microbiota, paving the way for the integration of soil microbial data into tools for disease monitoring and prediction in viticulture. All of these results, obtained from vineyards in the Bordeaux region, will need to be validated in other soil and climate contexts to establish their generalizability. ■

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*Source: Sourced from the research article: "Revealing microbial consortia that interfere with grapevine downy mildew through microbiome epidemiology" (Fournier et al., 2025).*

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