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Composting vine leaves to reduce the primary inoculum of *Plasmopara viticola* in the vineyard?

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Downy mildew caused by the oomycete *Plasmopara viticola* is one of the most damaging diseases affecting vineyards. The current issues surrounding the use of plant protection products are prompting the evaluation of protection strategies that include steps to prevent epidemic risks. The PROFIL project aims to extend the control period against this oomycete into the winter.

Reducing the primary inoculum of *Plasmopara viticola* through prophylactic measures

In autumn, sexual reproduction of *P. viticola* results in the formation of oospores in the leaves. Once on the ground, these overwintering forms can survive for several years and constitute the primary inoculum for future epidemics. Reducing the primary inoculum by exporting foliage from the vineyard after harvest (*e.g.* by uprooting or collecting it from the ground) is one way of limiting the accumulation of inoculum over time and reducing the pressure epidemic. One of the project's aims is to assess the effect of composting on the viability of oospores, so that sanitised organic matter can be returned to the vineyard.

Effect of high temperatures on the viability of *P. viticola* oospores

During the composting process, the organic matter is broken down by various physico-chemical processes and by the action of micro-organisms, resulting in an increase in temperature. During this so-called "thermophilic" phase, the temperature of the compost can exceed 70°C. Of all the reactions that can be observed in compost, the temperature effect is the easiest to control[1 2].

To assess the effect of high temperatures on the germinative capacity of oospores, vine leaves (Merlot grape variety) containing oospores were harvested in autumn 2021 and divided (in the form of leaf discs) into 15 samples (3 modalities and 5 replicates). For one month, 5 of the samples were placed in a humid oven at 50°C and 5 others in the same conditions, but at 70°C. The last 5, as controls, were kept in natural conditions on the soil for the same period. A bioassay was used to analyse the germinative capacity of the oospores as soon as they came out of the oven (March), one month later (April) and two months later (May): i) the leaves were placed in temperature and humidity conditions optimal for oospore germination (*i.e.*, 20°C darkness and immersion in water); ii) released zoospores are trapped using susceptible grapevine leaf discs (trap discs) and iii) oospore viability is assessed by characterising the degree of infection of leaf discs incubated under optimal sporulation conditions (*i.e.*, 20°C alternating 12 h day/12 h night, 100 % humidity)[3 4].

On average, 28.6 ± 4.2 % (standard deviation) of the control trap discs (n = 350) were infected. No infection was observed in the oven-dried samples (Figure 1, n = 700). These results therefore seem to indicate that *P. viticola* oospores lose their germinative capacity when they are exposed for 1 month to temperatures such as those encountered during the thermophilic phase of compost.

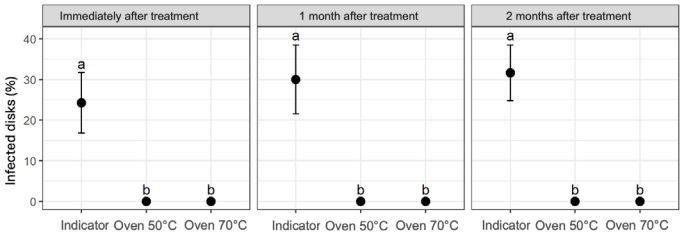


FIGURE 1. Percentage of infected trap discs used as an indicator of the germinative capacity of oospores contained in vine leaf samples, overwintered under natural conditions (control) or placed for 1 month in an oven at 50°C or 70°C. Different letters indicate significant differences.

Effect of temperature on oospore viability under actual conditions

Following this initial result under controlled conditions, the effect on the germinative capacity of oospores was evaluated under real conditions using a 50 m^3 compost composed mainly of vine shoots and grass cutting, turned and watered monthly. Vine leaves containing oospores were collected in the autumn of 2022, crushed and homogenised, then evenly distributed among 30 bags of blotting cloth (11 µm mesh, fine enough to hold the test material) and placed in the compost heap. These bags were buried in March 2023 at different depths to take account of vertical temperature stratification. Twelve samples were kept there for 3 months from March to May and twelve others for 4 months until June (4 repetitions per modality: duration x depth). Thermal readings were taken at various points on the 3 compost levels every 1 to 2 weeks (Figure 2). Six bags of leaf shreds from the same batch were placed in natural conditions on the ground near the compost heap, as controls (4 replicates per duration). The compost reached an average of 60°C at start-up and remained above 50°C for two months. Temperatures then fell, fluctuating between 37°C and 54°C depending on the zone, until the first samples were taken out in mid-May. In the compost core, the temperature never fell below 50°C. The temperatures reached are consistent with the temperatures expected in compost to give rise to its natural hygienisation[2].

The germinative capacity of oospores present in composted and control samples was assessed using the bioassay described above. No infection was observed on trap discs in contact with oospores placed in the compost (n = 960), whatever the duration and depth of burial (Figure 3). *In contrast*, for the control inoculum bags, 11.5 ± 3.98 % (standard deviation) and 2.84 ± 1.48 % (standard deviation) of the trap discs in May (n = 160) and June (n = 160)

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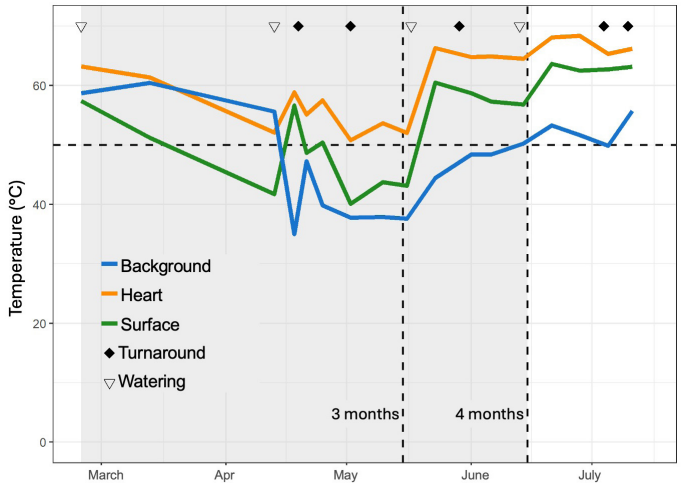


FIGURE 2. Changes in the average temperature of different layers of compost: at ground level - bottom (blue), in the core (orange) and on the surface (green) of the heap, from its installation in February 2023 to July 2023. The shaded period corresponds to the period during which the samples were present in the compost heap. The events of turning and watering of the compost heap are indicated by the black diamonds and white triangles respectively. The vertical dotted lines indicate the batch removal dates. The horizontal dotted line corresponds to 50°C, the minimum temperature tested under controlled conditions (oven).

respectively were infected (oospore germination rate known to decline after a peak in April). However, sporulation in the controls was lower than in the previous year due to a different initial inoculum, with mildew pressure being lower in 2022 than in 2021.

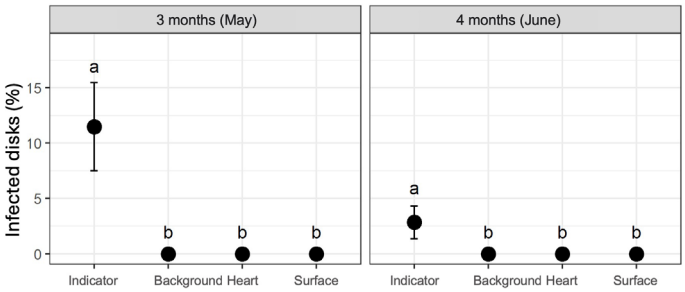


FIGURE 3. Percentage of infected trap discs as an indicator of the viability of oospores contained in vine leaf samples overwintered under natural conditions (control; n = 4) or placed for 3 or 4 months in compost at three depths: at the bottom of the pile (n = 4), in the centre of the pile (n = 4) or on the surface of the compost pile (n = 4).

The *P. viticola* oospores contained in vine leaves appear to lose their germinative capacity after spending 3 or 4 months in compost maintained at temperatures of between 40 and 70°C.

A promising prophylactic method that needs to be strengthened and integrated into an overall preventive strategy

Our trials showed an inhibition of the germination of *Plasmopara viticola* oospores after a period at high temperatures, both in controlled conditions and in actual composting conditions. Composting vine leaves extracted from plots in the autumn therefore appears to be effective in limiting the infectious power of the primary inoculum of downy mildew in grapevines, and the sanitised organic matter can be returned to the vineyard. Although the study focuses on the "temperature" parameter, the composition, intrinsic properties and management of the compost are essential points in controlling the success of this process for combating winter storage forms of downy mildew[1 5 6 7].

The vine leaf composting system, based on reducing the primary inoculum of *P. viticola*, could form part of an overall approach to the preventive management of mildew epidemics.

The development of a mechanical defoliation method for removing contaminated leaf biomass from significant areas in the autumn, and work on the effects on vine physiology and harvest quality are underway. A method for measuring downy mildew inoculum in the soil has already been developed[4] in order to assess the gradual clean-up of vineyard soils.

If this extraction-composting practice does indeed reduce epidemic pressure, it could limit the use of synthetic fungicides and make better use of protection strategies based on biocontrol products, which are still more difficult to control than traditional fungicides, while encouraging the composting of shoots (rather than burning them) and contributing to the organic fertilisation of vineyards. ■

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