SUPPLEMENTARY DATA

S1. Protocol

This protocol describes the procedure for establishing small-scale *Plasmopara viticola* infections on living grapevines. The workflow is outlined using a single leaf for pre-infection propagation and a single potted grapevine for infection. The design can be scaled to larger sample sizes as needed.

Materials/equipment and environment conditions

The following materials and equipment were used:

- Bovine serum albumin (BSA)
- Calcium nitrate
- Distilled water
- Filter paper
- Incubator
- Climate chamber or poly tunnel with fog nozzles
- Tweezers
- Scalpel
- Spray bottle (*e.g.*, 20 mL capacity)
- Petri dishes (150 mm diameter, with lids)
- Potted grapevines
- *Plasmopara viticola* sporangia (fresh or frozen).

The following environmental conditions were necessary for successful implementation:

- Incubator: 16-hour photoperiod (long-day conditions) at 18 °C and 60 % relative humidity; and
- Climate chamber/poly tunnel: 16-hour photoperiod at 22–24 °C, 40–60 % relative humidity during the light phase; 18 °C and 85–98 % relative humidity during the dark phase.

Pre-infection phase

1. Preparation of propagation material (duration: minutes)

- Prepare the propagation solution as described and store it in a tightly sealed bottle (at 8 °C, stable for two weeks);
- Detach a fresh grapevine leaf from a potted plant and carefully rinse both surfaces with distilled water. Dry it afterwards with *e.g.* soft paper towels; and
- Prepare the sporangia suspension by dispensing 35 mL of propagation solution (2 µg/mL BSA (Merck Darmstadt, Germany, cat nº 1.12018.0025) and 0.42 µg/mL $Ca(NO_3)_2$*4 H_2O (Carl Roth Karlsruhe, Germany, cat nº P740.4)) into a Petri dish and adding a small amount of fresh or frozen sporangia (*e.g.*, a spatula tip – there is no need to prepare a suspension with a known number of spores). Alternatively, submerge a previously propagated leaf inoculum, sporangia-side down, into the propagation solution for 10 minutes to release zoospores into the liquid.

2. *In vitro* propagation (duration: five–six days)

- Inoculate the cleaned grapevine leaf by placing it abaxial side down into the sporangia suspension;
- Close the Petri dish and incubate in darkness for 24 hours, covering the dish with aluminium foil or another opaque material in an incubator (see environmental conditions);

- After incubation, remove the leaf, place filter paper in the Petri dish to absorb excess liquid. Place the leaf back in the Petri dish, on top of the filter paper maintaining the lower surface facing upwards;
- Maintain high humidity by keeping the Petri dish closed and continue incubation in the incubator with a 16-hour photoperiod (long-day conditions) and at 18 °C and 60 % relative humidity; and
- Sporulation typically occurs within four to five days. Newly formed sporangia can be used directly for infection or to prepare a fresh sporangia suspension for further propagation.

Infection phase

3. Preparation of infection (duration: minutes)

- Clean the target leaf for inoculation on the potted grapevine with distilled water and allow it to dry completely. Mark the leaf base using adhesive tape or a comparable marker (see Figure S1);
- Transfer the potted grapevine to a climate chamber, polytunnel or other controlled growth environment;
- Assess the sporangia formation on the *in vitro* culture (see Figure 2);
- Excise a small fragment (~5 mm^2) from the sporulating leaf using a scalpel, avoiding major leaf veins to ensure even surface contact; and
- Carefully transfer the fragment with tweezers, placing it abaxial side up onto a moist filter paper within a separate Petri dish to avoid it drying.

4. Sporangia transfer (duration: eight hours)

- Using a spray bottle, gently apply propagation solution to the sporangia-covered surface of the leaf fragment;
- Attach the moistened fragment to the lower surface of a leaf on the potted grapevine. The liquid film generates adhesive forces, enabling attachment without external support (Figure S1);
- Maintain the potted grapevine in a climate chamber, polytunnel or other controlled growth environment, starting with an eight-hour dark phase; and
- Remove the leaf fragment immediately after the dark phase to minimize the risk of secondary contamination.

Post-infection phase

5. Incubation and observation (duration: four–seven days)

- The inoculated plants are incubated up to seven days in a climate chamber, polytunnel or other controlled growth environment under the day and night time conditions described above (see environmental conditions);
- Visual signs of sporulation typically become evident four–seven days post-infection; and
- Monitor the inoculated area daily. Expansion of the infection site and leaf decolouration may occur in subsequent days after infection is first observed.

SUPPLEMENTARY DATA
Kleb, M., Zipper, R., Merkt, N., & Zörb, C. (2026). Artificially induced and precise small-scaled infection with *Plasmopara viticola* on living vines. *OENO One*, *60*(1).
https://doi.org/10.20870/oeno-one.2026.60.1.9395

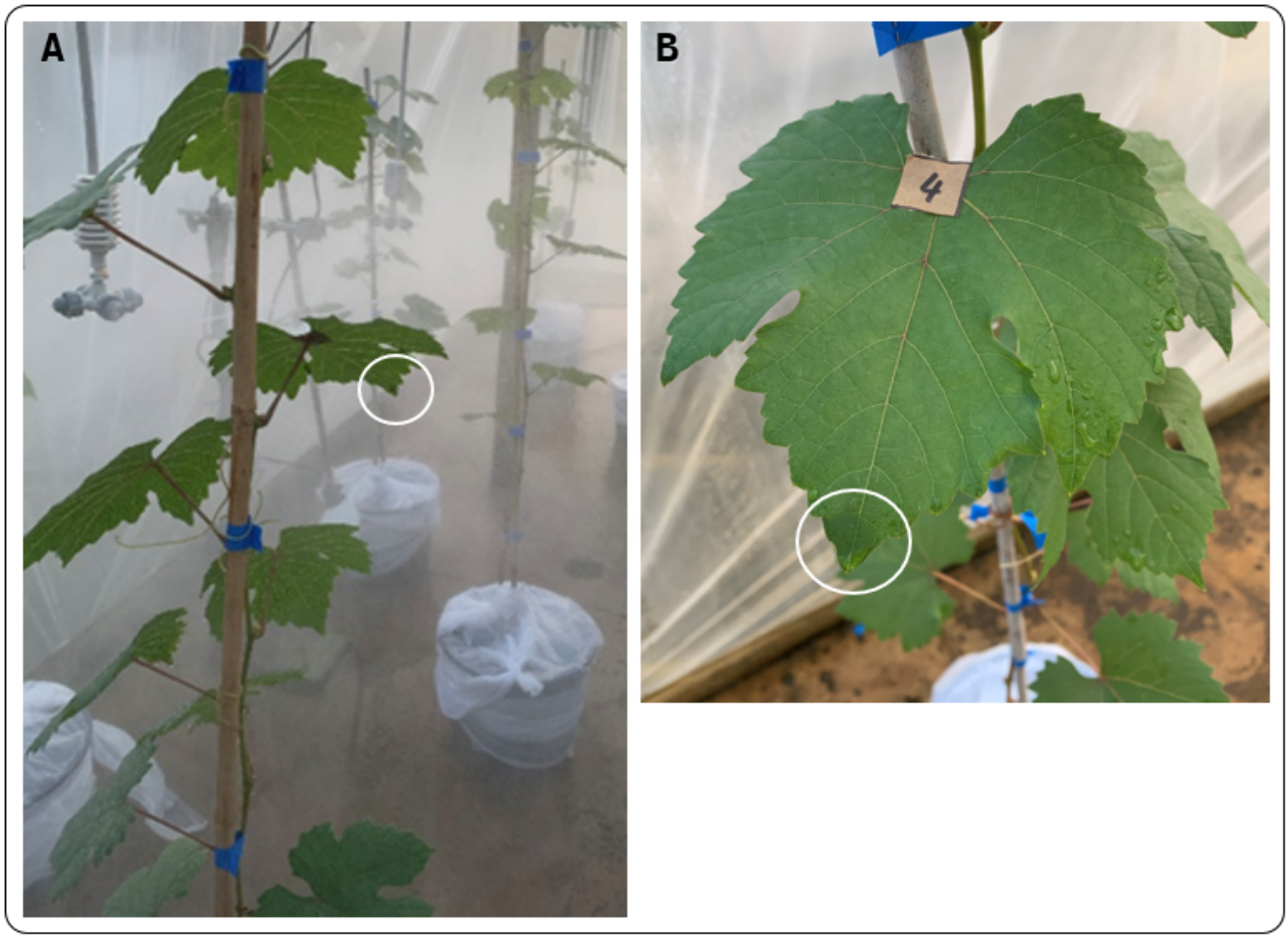


Figure S1. Leaf fragment of the inoculum, attached to the lower leaf surface during the infection procedure. (A) Potted grapevines in a poly tunnel at high humidity; (B) Close-up of the upper leaf surface with the emerging shadow from the inoculum fragment.